EPIDEMIOLOGY OF NATURAL TRANSMISSION OF BOVINE LEUKEMIA VIRUS INFECTION

BY

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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A 27-month study examined 473 dairy cattle for associations between bovine leukemia virus (BLV) infection and host and environmental factors. Cattle sera were tested at monthly intervals for BLV antibodies by agar-gel immunodiffusion using the glycoprotein-51 antigen. A model of BLV colostral antibody decay in 130 calves predicted infection in calves less than six months of age and estimated antibody half-life to be 27.1 ± 1.2 days. Colostral antibody decay did not differ between BLV-infected and noninfected calves for slope ($p = 0.45$) or intercept ($p = 0.43$). By 95 days of age, 50% of the calves had no detectable BLV colostral antibodies.

Of 125 calves born to BLV-infected cows and followed for at least four months, eight (6.4%) had precolostral BLV antibodies, as determined by radioimmunoassay using the glycoprotein-51 antigen. In utero infection with BLV was

not associated with dam age ($p = 0.86$), dam parity ($p = 0.83$), breed ($p = 0.66$), sex ($p = 0.11$), or stage of gestation in which the dam was infected ($p = 0.50$). Calves infected *in utero* did not pose an increased risk of infection to calves penned next to them ($p = 0.61$).

Prevalence rates of infection were 9%, 16%, and 63% at 6, 16, and 27 months of age, respectively. Age-specific rates of infection were not associated with dam age ($p = 0.79$), dam parity ($p = 0.75$), dam BLV-status ($p = 0.46$), breed ($p = 0.86$), or BLV-status of colostrum consumed ($p = 0.50$).

An algorithm was described which allocated probabilities of infection to locations occupied by an animal prior to detection of infection. Small calf pastures were associated with less infection than was the calf barn ($p < 0.05$). No less infection was associated with individual outdoor calf pens compared to contiguous indoor pens ($p > 0.05$). Risk of infection associated with the dry herd was five times that for heifer pastures ($p < 0.0001$) and accounted 24 infections per 100 heifers per 100 days at risk.

Vaccination for infectious diseases was not associated with increased BLV infection ($p = 0.33$). Infection rates were not associated with month of birth ($p = 0.24$) or with season of potential arthropod vectors ($p = 0.20$). Heifer infection was likely to occur in late winter or spring ($p = 0.01$).

CHAPTER I
INTRODUCTION

Bovine leukemia virus (BLV) has been shown to be the causative agent of enzootic bovine leukosis, a neoplastic disease of cattle (Callahan et al., 1976; Kettmann et al., 1976; Miller et al., 1969). Bovine leukosis is believed to have spread to western European countries from the Baltic region during World War I (Bendixen, 1965). Following World War II, efforts were undertaken in some European countries to reduce the tumor incidence rate through hematologic examinations for persistent lymphocytosis, a phenomenon associated with bovine lymphosarcoma (Bendixen, 1965). After discovery of BLV, and subsequent development and use of serologic methods for mass screening (Hoff-Jorgensen et al., 1978; Miller et al., 1969; Miller and Van Der Maaten, 1976a; Onuma et al., 1975; Schmidt et al., 1978), eradication of enzootic bovine leukosis progressed rapidly (Bause et al., 1978; Mammerickx et al., 1978a; Straub, 1978b).

In order to preserve gains made in these programs, restrictions were placed on BLV-seropositive cattle and on semen entering countries either free from BLV or with BLV control programs (Miller, 1980). Such restrictions have placed an economic burden on the cattle export markets of the United States (Mix, 1979). Because of the high genetic

quality of American cattle, eradication or control of BLV infection using European methods of test and slaughter would not be a pragmatic alternative for the American producer. Interest, therefore, has focused on prevention of transmission and test and segregation within a herd (Miller and Van Der Maaten, 1978a).

A prerequisite to control of BLV in a herd is a clear understanding of natural transmission of infection from fetal life to adulthood or to the age at which heifers would move to export markets. Several constraints make the study of natural transmission patterns difficult and may explain the lack of reports of long-term prospective studies in the literature. A major obstacle is the necessity for a large sample of animals to be tested at close intervals over a long time period. At the same time changes in management or environmental factors must be recorded.

The device used to measure infection must be sensitive, specific, inexpensive, simple, and meet specifications of other programs. This necessitates the use of agar-gel immunodiffusion because it fulfills the above conditions (Miller, 1980). Definition of infection by a serologic test, however, has important limitations. For instance, discrimination has not been made between colostral antibodies and infection-induced antibodies in calves less than six to seven months of age which consumed colostrum from a BLV-infected cow (Ferrer et al., 1977b). Another problem in defining infection is that seroconversion may

lag behind BLV infection by as much as two to three months (Mammerickx et al., 1980; Straub, 1978b; Van Der Maaten and Miller, 1978b, 1978c).

These constraints are not unique to the study of the epidemiology of BLV transmission. It is important, therefore, that designs for the study and eventual control of BLV be generally applicable to the study of other diseases and infections.

Examination of risks of BLV infection in a large cattle population over a 27-month period is presented here as a logical progression from the fetal environment to adulthood. Detection of BLV infection based on serologic criteria is used as a proxy for infection. The intent is not to attempt statements about specific routes of BLV infection, but to describe temporal and spatial patterns of natural infection observed in animals studied. Furthermore, factors possibly associated with deviations in those patterns will be examined using existing analytic methods in a framework applicable to other diseases. In addition, new techniques are presented which improve the efficiency of a serologically-based diagnosis.

CHAPTER II
LITERATURE REVIEW

Bovine Leukemia Virus Infection

Clinical Appearance

Manifestations of infection with BLV vary from no signs to persistent lymphocytosis or to lymphosarcoma (Abramova et al., 1974; Grimshaw et al., 1979; Kenyon and Piper, 1977; Kumar et al., 1978; Sorenson, 1979; Stober, 1968). Clinical signs of tumor involvement usually are seen in cattle over five years of age and are referable to the organ system involved (Abramova et al., 1974, Grimshaw et al., 1979; Sorenson, 1979; Stober, 1968).

Sporadic bovine leukosis (i.e., juvenile, thymic, or cutaneous leukosis) is not associated with BLV infection (Bundza et al., 1980; Chander et al., 1977; Onuma, 1978; Onuma et al., 1979; Richards et al., 1981; Straub and Weiland, 1977).

In the absence of tumor involvement, BLV-infected animals do not appear to suffer production losses (Langston et al., 1978).

Distribution

Bovine leukemia virus infection is a ubiquitous infection throughout the world (Burny et al., 1980; Burridge et al.,

1981). A survey of cattle in the state of Florida recently estimated the infection rate of BLV in dairy cattle to be 48% (Burridge et al., 1981).

Bovine Leukemia Virus

Discovery of BLV was made following phytohemagglutinin-stimulation of lymphocytes from cattle with lymphosarcoma (Miller et al., 1969). The virus is classifed as a single-stranded RNA retrovirus (Burny et al., 1980). It is spherical in shape with a diameter of 60-125 nm (Calafat et al., 1974; Calafat and Ressang, 1977a, 1977b; Dutta et al., 1970; Miller et al., 1969).

Several viral proteins have been described. There are at least two glycoproteins, gp-30 (Dietzschold et al., 1978) and gp-51 (Onuma et al., 1975), which constitute the outer shell of BLV (Burny et al., 1980; Devare and Stephenson, 1977; Driscoll et al., 1977). An ether-resistant protein constitutes the internal or core antigen, known as p-24 (Gilden et al., 1975; McDonald and Ferrer, 1976; Miller and Olson, 1972). The BLV genome codes for a reverse transcriptase which has a unique requirement for Mg^{++} (Dietzschold et al., 1978; Gilden et al., 1975; Graves et al., 1977; Kettmann et al., 1976). Six mutant strains of BLV have been investigated recently (Couez et al., 1981; Kettmann et al., 1981).

Several investigators have identified BLV as a C-type virus (Burny et al., 1980; Dutta et al., 1970; Ferrer et al.,

1971; Kawakami et al., 1970; Mussgay et al., 1977; Stock and Ferrer, 1972; Van Der Maaten et al., 1974; Weiland and Ueberschar, 1976). Others have been reluctant to describe BLV as a B- or C-type virus since immature viruses are rarely found outside the cell (Calafat et al., 1974; Calafat and Ressang, 1977a, 1977b; Dekegel et al., 1977).

Comparisons of BLV with other retroviruses or oncoviruses by molecular hybridization have shown that BLV is biochemically distinct from Friend mouse leukemia virus and visna maedi virus (Kaaden et al., 1977), Rauscher leukemia virus (Kettmann et al., 1975, 1976), simian sarcoma (wooly monkey) virus, murine sarcoma virus, feline sarcoma virus, and feline leukemia virus (Kettmann et al., 1975, 1977). Other studies have demonstrated a lack of cross-reactivity between proteins of BLV and Mason Pfizer monkey virus (McDonald and Ferrer, 1976; McDonald et al, 1976), and bovine syncytia virus (McDonald et al., 1976).

Pathogenesis

Tissues Involved

Replication of BLV occurs mainly in B-lymphocytes (Kenyon and Piper, 1977; Paul et al., 1977), but an association with T-lymphocytes also has been reported (Takashima et al., 1977). Further support for B-cell involvement was found in the expansion of the B-cell population in BLV-infected cattle (Kenyon and Piper, 1977; Kumar et al., 1978).

Following subcutaneous inoculation of leukocytes from a BLV-infected steer, BLV was isolated from the spleen after eight days, from leukocytes after 14 days, and occasionally from prescapular lymph nodes thereafter (Van Der Maaten and Miller, 1978b). In that study, BLV could be isolated from lymphocytes two to three weeks before a detectable serologic response, and virus was not isolated from the thymus. As few as 2500 washed lymphocytes from an infected steer have been able to transmit BLV infection to susceptible calves (Van Der Maaten and Miller, 1978c).

Integration of BLV in the Host Cell Genome

Results of studies using BLV-specific DNA probes suggest that BLV is an exogenous virus (Callahan et al., 1976; Deschamps et al., 1981; Kettmann et al., 1976, 1978a, 1978b, 1979a; Kukaine et al., 1979). The DNA from lymphocytes of BLV-infected cattle has viral sequences that cannot be identified in the DNA from lymphocytes of noninfected cattle (Callahan et al., 1976; Kettman et al., 1976), or in normal cell DNA from BLV-infected cows (Kettmann et al., 1978a).

The BLV provirus is integrated in several sites of the lymphocyte DNA in cattle with persistent lymphocytosis, but in only one or a few sites in the DNA of cells of lymph node tumors (Kettmann et al., 1979a, 1980a, 1980b). Less than 5% of peripheral lymphocytes in asymptomatic, infected cattle contain the provirus, whereas up to 33% of

the circulating lymphocytes in cattle with persistent lymphocytosis contain the provirus (Kettmann et al., 1980b).

Seroconversion Period

Seroconversion following infection with BLV has been found to occur between two and seven weeks in cattle experimentally inoculated (Mammerickx et al., 1980; Van Der Maaten and Miller, 1978a, 1978b). Half of the animals in these studies had seroconverted by five weeks postinoculation. The seroconversion period was similar for sheep experimentally inoculated by the intradermal route, oral route, or by BLV-carrying tabanid flies (Gentile and Rutili, 1978; Mammerickx et al., 1980; Ohshima et al., 1981). The seroconversion period for animals naturally infected is considered to be less than three months (Straub, 1978b), and the pattern of seroconversion is believed to be similar to that for experimentally infected animals (Van Der Maaten and Miller, 1978b).

Transmission Via Excretions and Secretions

It is well documented that BLV can be experimentally transmitted to cattle via blood (Mammerickx et al., 1980; Van Der Maaten et al., 1981a) and lymphocytes (Miller and Van Der Maaten, 1978b; Van Der Maaten and Miller, 1978a, 1978b) from infected animals. The virus has been demonstrated in saliva but not in prostatic fluid or feces of infected cattle (Ressang et al., 1980). The p-24 antigen

of BLV has been found in urine of naturally infected animals (Gupta and Ferrer, 1980).

Semen collected by manual massage from a BLV-infected bull transmitted BLV infection to susceptible sheep (Lucas et al., 1980). Another study failed to demonstrate BLV in semen collected from BLV-infected bulls following normal ejaculation (Miller and Van Der Maaten, 1979).

Serology

Several serologic tests for the detection of BLV have been described for both the gp-51 and the p-24 antigen (Burny et al., 1980). Agar-gel immunodiffusion using gp-51 has been recommended for use by member countries of the European Economic Community (Kaaden and Stephenson, 1978), because of its high sensitivity, simplicity, and low cost (Miller, 1980). Recently a radioimmunoassay procedure was described using gp-51 (Bex et al., 1979). This test may be the most sensitive one presently available (Miller et al., 1981).

Factors Examined for Associations with Bovine Leukemia Virus Infection

Genetic Susceptibility

A genetic predisposition to enzootic bovine leukosis and bovine lymphosarcoma was suspected before discovery of BLV. Leukosis was observed more frequently in daughters of affected cows than in daughters of unaffected cows

(Bendixen, 1965; Larson et al., 1970). Pedigree studies of lymphosarcoma found clustering of cases by sire and/or dam families (Crowshaw et al., 1963; Cypess et al., 1974; Marshak et al., 1962). It also was observed that herds which were inbred experienced higher rates of leukosis and lymphosarcoma than did noninbred herds (Abt, 1968; Laktionov and Nakhmanson, 1972), but purebred herds were found to have lower rates of leukosis than nonpurebred herds (Anderson et al., 1971).

One study estimated the heritability of susceptibility to BLV infection to be 0.44 ± 0.22 (Burridge et al., 1979). A study of lymphosarcoma, however, was not able to associate the disease with serologically defined antigens controlled by the BoLA-A locus (Takashima and Olson, 1978).

Parental Infection with Bovine Leukemia Virus

The effect of BLV infection of the dam on subsequent BLV infection in the offspring has been examined by several investigators. In one report, dam status appeared to have a significant influence on progeny infection (Baumgartener et al., 1978), but the authors felt that such an effect may have been due to high prevalence rates in some herds. In a longitudinal study, no association was found between dam status and age at which progeny became infected (Olson et al., 1978). Reports on cross-sectional studies concluded that presence of BLV antibodies in the dam was not associated with subsequent progeny infection (Hofirek, 1980; Valikhov, 1978).

In a large study of progeny from BLV-infected, AI bulls, offspring from infected sires did not have as high a rate of subsequent BLV infection as did those from noninfected sires (Baumgartener et al., 1978).

Sex

Few studies have examined for associations between BLV infection and sex. Reports in which sex effects were studied suggested no difference in infection rates between males and females (Baumgartener et al., 1975; Evermann et al., 1980).

Breed

It has been reported that many different breeds are susceptible to BLV infection (Burridge et al., 1981; Marin et al., 1978). Analysis of data from a survey of Florida cattle suggested that Jerseys had a higher infection rate than Holsteins (Burridge et al., 1981). However, a study within a Florida dairy herd indicated no difference existed between rates of infections for Jerseys and Holsteins (Burridge et al., 1979).

Age

Age-specific prevalence rates of BLV infection have been shown to follow a characteristic sigmoidal curve. Rates increased linearly from one to four years of age, after which they plateaued (Burridge et al., 1979, 1981;

Chander et al., 1978; Evermann et al., 1980; Ferrer et al., 1976; Hofirek, 1980; Mammerickx et al., 1978a, 1978b; Marin et al., 1978; Olson et al., 1973; Piper et al., 1979). Peak prevalence rates of infection were observed at four years of age in beef cattle and at more than nine years of age in dairy cattle (Burridge et al., 1981). Ages at which a sharp, linear increase in rates occurred varied from study to study and from herd to herd. In some herds rates of infection began to level off at two to four years of age (Burridge et al., 1979; Olson et al., 1973), while in other herds rates reached a plateau at four to five years of age (Chander et al., 1978; Hofirek, 1980; Mammerickx et al., 1978a, 1978b; Marin et al., 1978).

A few studies have approached age-specific rates of infection in a longitudinal design using birth cohorts. Results of one of these investigations showed that animals of similar ages experienced different rates of infection, according to the birth cohort (Wilesmith et al., 1980). In the other study, each new 12-month cohort entered the herd with a lower prevalence rate than did the previous cohort. Rates within a cohort did not appear to change as cattle aged. It was suggested further that higher prevalence rates of infection observed in older animals in cross-sectional studies represented high-rate cohorts (Huber et al., 1981).

Transmission of Bovine Leukemia Virus Infection

In Utero

Rates of natural in utero infection with BLV have been reported to range from 3% to 25% (Ferrer et al., 1976, 1977a, 1977b; Piper et al., 1979). Stage of gestation during which a dam is experimentally infected has not been associated with the frequency of infection in progeny (Van Der Maaten et al., 1981b).

Physical Contact

Close physical contact between infected and susceptible cattle is believed to be a prerequisite to BLV transmission (Ferrer and Piper, 1981; Maas-Inderwiesen et al., 1978; Miller and Van Der Maaten, 1978a; Wilesmith et al., 1980). Newborn calves were more likely to develop leukosis when placed in close contact with leukotic cows (Straub, 1971). Infection rates increased during winter months in one herd studied, suggesting transmission associated with indoor housing conditions (Wilesmith et al., 1980). Limiting physical contact by vacating a stall between animals or by placing a single fence between animals appeared to retard transmission of infection (Miller and Van Der Maaten, 1978a).

Arthropod Vectors

Bovine leukemia virus has been isolated from the midgut of horseflies after feeding on a BLV-infected cow

(Bech-Nielsen et al., 1978). In an experimental study, horsefly transmission of BLV infection to sheep was demonstrated (Ohshima et al., 1981). It has been suggested that high rates of infection observed in animals during summer months support the hypothesis of vector-borne transmission of BLV infection (Bech-Nielsen et al., 1978; Onuma et al., 1980). Another study, however, observed higher rates of infection during winter months (Wilesmith et al., 1980).

Ixodes ricinus ticks have been suggested as a possible explanation for geographic differences in rates of infection in Sweden (Hugoson and Brattstrom, 1980).

Aerosol

Intranasal instillation of BLV-infected lymphocytes produced infection in one of two calves and an aerosol exposure to BLV-culture fluids produced infection in two of two calves (Van Der Maaten and Miller, 1978c). Both methods, however, also exposed the oral cavity and the latter method exposed the conjunctivae.

Oral

Bovine leukemia virus or BLV-like particles have been identified in milk and colostrum of BLV-infected cows and cows with lymphosarcoma (Dutcher et al., 1964; Jensen and Schidlovsky, 1964; Miller and Van Der Maaten, 1979; Schulze et al., 1966). Transmission of BLV by the oral

route has been demonstrated by inoculation of leukemic blood into neonatal lambs (Mammerickx et al., 1976a), by inoculation of lymphocyte cultures into colostrum-deprived calves (Miller et al., 1972; Van Der Maaten and Miller, 1978c), and by feeding BLV-infected lymphocytes in colostrum free of BLV antibodies (Van Der Maaten et al., 1981a). Oral transmission has not been shown in old lambs (Hoss and Olson, 1974) or in old calves (Van Der Maaten and Miller, 1978c). Infection did not occur when calves were fed BLV-infected lymphocytes in colostrum containing BLV antibodies (Van Der Maaten et al., 1981a). Some studies concluded that considerable emphasis should be placed on oral transmission, especially when bloody colostrum or milk is fed (Parfanovich et al., 1978; Seger and Morgan, 1977). Results of other studies suggest that milk or colostrum does not constitute a major vehicle for transmission of BLV infection (Ferrer et al., 1976; Ferrer and Piper, 1978, 1981; Piper et al., 1975, 1979). Pasteurization of milk or colostrum for 30 seconds at 60°C would probably inactivate the virus (Baumgartener et al., 1976).

Venereal

One study demonstrated the venereal transmission of BLV infection after placing BLV-infected lymphocytes in the cervical canal of susceptible cows (Van Der Maaten and Miller, 1978c).

Iatrogenic

Intradermal inoculation of BLV has been shown to be a viable means of transmission (Van Der Maaten and Miller, 1978c). Iatrogenic transmission of BLV following blood sampling procedures has been suspected (Bause et al., 1978; Maas-Inderwiesen et al., 1978; Wilesmith, 1979). Premunization of cattle for babesiosis and other vaccination procedures also have been incriminated in BLV transmission (Hugoson and Brattstom, 1980; Hugoson et al., 1968; Marin et al., 1978; Stamatovic and Jonavic, 1968). However, transmission of BLV to sheep following intradermal Tb testing has not been demonstrated (Roberts et al., 1981).

Infection in Other Domestic Animals

The possibility for BLV infection in species other than the bovine was first suggested when bovine leukosis and later bovine lymphosarcoma were transmitted to sheep (Olson et al., 1972; Wittmann and Urbaneck, 1969). Experimental infection with BLV has been confirmed in sheep (Bansal and Singh, 1980; Bex et al., 1979; Gentile and Rutili, 1978; Hoss and Olson, 1974; Mammerickx et al., 1976a, 1976b, 1980, 1981; Olson and Baumgartener, 1978; Van Der Maaten and Miller, 1976a), in goats (Hoss and Olson, 1974; Mammerickx et al., 1981; Olson et al., 1981), and in pigs (Mammerickx et al., 1981). Evidence exists for experimental BLV infection in rabbits, but not in rats

(Bansal and Singh, 1980), and in guinea pigs (Lussier and Pavilanis, 1969). Serologic evidence for BLV infection in chimpanzees has been found (Van Der Maaten and Miller, 1976b). Passage of BLV through cattle, sheep, or goats did not reduce the infectivity of the virus for cattle, sheep, goats, or pigs (Mammerickx et al., 1981). Sheep have not been found to shed BLV and thus may not be a reservoir (Mammerickx et al., 1976b; Van Der Maaten and Miller, 1976a).

Reviews

Bovine leukosis, bovine lymphosarcoma, and bovine leukemia virus infection have been extensively reviewed (Bendixen, 1965; Burny et al., 1978, 1980; Ferrer, 1977, 1979, 1980a, 1980b; Ferrer et al., 1978, 1979; Hoff-Jorgensen, 1977; House et al., 1975; Markson, 1979; Mussgay and Kaaden, 1978; Olson, 1974; Olson et al., 1970; Reed, 1981; Ruppanner and Paul, 1980; Tyler, 1978; Van Der Maaten and Miller, 1975).

CHAPTER III
GENERAL MATERIALS AND METHODS

Population Studied

Cattle studied were those in the University of Florida Dairy Research Unit (DRU) herd. This population was chosen because it had been characterized previously by a BLV prevalence rate of 75% (Burridge et al., 1979). Calves born from July 1, 1979, through June 30, 1981, were followed until death, sale, parturition, or September 30, 1981, whichever occurred first.

Location and Climate

The DRU was located in north-central Florida (latitude 82°30' west, longitude 29°40' north). It occupied about 450 hectares of reclaimed pine flatwoods 20 km. northwest of the University of Florida at Gainesville, Florida (APPENDIX A). The climate was characterized by hot, humid summers and cool, dry winters (APPENDIX B).

Management Practices

The DRU was a closed herd used as a research facility. Two 18-month-old heifers, a Jersey (M101) and a Holstein (M121), entered the herd, however, from a commercial dairy

(Bassett's Dairy, Montecello, FL) on May 5, 1980. Both were negative for BLV antibodies when they entered the herd. The Jersey had BLV antibodies by April 25, 1981, and the Holstein remained serologically negative through September 26, 1981.

Approximately 200 purebred cows were milked, 1/3 of which were Jerseys and 2/3 Holsteins. Cattle were allowed to graze, but it was necessary to supplement rations with corn silage; corn, sorghum, ryegrass, or alfalfa greenchop; and concentrates.

Cattle were bred artificially using commercially available frozen semen. Some semen was used, however, from young Jersey control sires raised at the DRU. Calving season ranged from June through December (APPENDIX C). Calves were born in small pastures (APPENDIX D, Fig. 1) and remained with their dams for about 12 hours. They then were placed in either contiguous, wire pens in the calf barn (APPENDIX D, Fig. 2) or in individual outdoor pens (APPENDIX D, Fig. 3). Once in pens, they were fasted for 12 to 24 hours, after which time they were fed either pooled colostrum (obtained from cows one through three days postpartum) or bulk-tank milk for the next two to three days. Prior to weaning at about one month of age, calves were fed bulk-tank milk once a day. Holsteins received 9 lbs. per day and Jerseys 7 lbs. per day. Calves also were fed concentrates ad libitum from two or three days of age.

Electric dehorning, ear tagging, and tattooing were performed during the first month of life. This usually was not done in a serial fashion from calf to calf at one session, but individually, one or two calves per day.

All heifers were vaccinated for brucellosis,[a] clostridial diseases,[b] and leptospirosis[c] at four months of age and for infectious bovine rhinotracheitis (IBR),[d] bovine virus diarrhea (BVD),[d] and parainfluenza-3 (PI-3)[d] at 11 months of age. Brucellosis vaccination was administered subcutaneously in the neck and other vaccinations intramuscularly in the gluteal area. Cattle were vaccinated in groups using one or two common 18 gauge 1-1/2 inch needles and a multidose syringe. The order in which cattle were vaccinated was not recorded.

At the time of brucellosis vaccination, calves were wormed either with thiabendazole[e] or levamisole.[f] The specific type administered to each calf was not recorded.

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[a]Strain 19, Colorado Serum Co., 4950 New York St., Denver, CO.

[b]Siteguard, Clostridium chauvoei, septicum, haemolyticum, novyii, sordellii, perfringens Type C and D. Jensen-Salsbery, Kansas City, MO.

[c]Novalep GHP, Jensen-Salsbery, Kansas City, MO.

[d]RESBO 3, Norden Laboratories Inc., Lincoln, NE.

[e]Omnizole (paste), Merck Animal Health Division, Merck & Co., Inc., Rahway, NJ.

[f]Ripercol L, American Cyanamid Co., Princeton, NJ, or Levasole, Pitman-Moore, Inc., Washington Crossing, NJ.

At two to three months of age, calves were moved from indoor pens in the calf barn, or from nearby outdoor pens, to paddocks (APPENDIX D, Fig. 4) or to small pastures (0.3 to 0.6 ha.) (APPENDIX D, Fig. 5). At about 10 months of age, they were moved to larger pastures (2 to 3 ha.) (APPENDIX D, Figs. 6 and 7). Cattle in each location were of similar ages.

Heifers were bred between 13 and 16 months of age, except during summer months when estrous behavior was less apparent. At 40-45 days gestation, heifers were placed, for the first time, with adult cattle in the dry herd. For heifers under study, this began in early January, 1981. Holstein heifers were removed from the dry herd on May 1, 1981, and placed in pastures holding only bred Holstein heifers. Jersey heifers continued to be placed in the dry herd and remained there until shortly before calving.

Most bull calves were sold a few days after birth, although some were retained for veal studies or semen collection. Management of heifers was similar to that practiced on most commercial dairies. Heifers usually were not used in experimental studies until after freshening.

Fly control was practiced sporadically two or three times during summer months. Lactating cows were hand-dusted with a commercial insecticide,[a] and fly bait[b] was

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[a]Marlate 50, E. I. DuPont de Nemoud and Co., Inc., Wilmington, DE.

[b]Golden Malrin, Starbar Division of Zoecon Corp., 12200 Denton Dr., Dallas, TX.

scattered in the calf barn a few times during peak calf density in the fall. Pastured heifers were not treated for flies. Most flies observed in the calf barn were Musca domestica feeding on decaying grain and soured milk. Pastured cattle were bothered by large numbers of Haematobia irritans and Stomoxys calcitrans and some tabanidae. No ticks or lice were observed during the 27-month study.

Sampling Design

Precolostral blood samples were drawn on nearly all calves born alive between July 1, 1979, and September 30, 1980. To increase the chance of obtaining precolostral samples, udder bags[a] were placed on cows about one week prior to calving (APPENDIX D, Fig. 1). This prevented the calf from nursing until a blood sample could be drawn. In order to reduce the period of colostrum deprivation, research field personnel were notified by telephone as soon as a cow calved. This procedure was instituted in December 1979, and calves born after this time were fed 1-2 liters of colostrum from their own dam within one hour following birth. After feeding, the navel was washed with a solution of 3% Lugol's iodine. Through December 1979, calves were bled precolostrally, at one week of age, and then at three-month intervals. After December 1979, calves were bled precolostrally, at two to three days of age, and then at

[a]Numbers C4591N-C4593N, Nasco, Fort Atkinson, WI.

monthly intervals beginning some time during the first month of age. Calves born between September 30, 1980, and June 30, 1981, were not bled precolostrally, but were bled at monthly intervals beginning sometime during the first month of age. Calves born after June 30, 1981, were not included in the study. Cows were bled at two, five, and eight months of gestation, at parturition, and at one month postpartum. All blood samples were collected from either the jugular or intercoccygeal vein using a sterile 20 gauge 1-1/2 inch needle[a] and Vacutainer tube.[b]

Demographic Information

The following information was obtained for each calf: BLV status of dam, age of dam, parity of dam, breed, sex, birth date, sale date, death date, or freshening date. Locations and inclusive dates of occupation in pens and pastures were recorded for all cattle as long as they were followed. As soon as heifers began entering the dry herd, all cattle in that group were sampled monthly to determine the BLV status of animals exposed to heifers being studied. No interventions were made in routine management of calves or heifers except for colostrum feeding, navel washing, and monthly mustering and bleeding.

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[a]No. 5746, Becton-Dickinson and Co., Rutherford, NJ.

[b]No. 6512, Becton-Dickinson and Co., Rutherford, NJ.

Serology

Serum samples were tested for presence of antibodies to BLV glycoprotein-51 antigen (gp-51)[a] using agar-gel immunodiffusion (AGID), as previously described (Burridge et al., 1979). Gel plates were incubated at 24-27 C in an humidified chamber for 48 hours before being read with the aid of a direct light beam.[b] Formation of precipitation lines of identity with positive control sera indicated presence of BLV antibodies (APPENDIX E). Serial two-fold dilutions using phosphate buffered saline[c] were made of all positive sera and then these dilutions were retested to determine end-point titers.

All precolostral sera from calves lost to follow-up before four months of age and negative on AGID were retested using a radioimmunoassay (RIA) procedure with gp-51 (Bex et al., 1979).[d] Samples precipitating more than 15% of labeled antigen were considered positive for BLV antibodies. Persistence of antibodies or an increase in BLV antibody titer was considered evidence of BLV infection. Precolostral sera from calves with evidence of BLV infection at some later date were retested by RIA.

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[a]Antigen supplied in the Leukassay-B kit, Pitman-Moore, Inc., Washington Crossing, NJ, and by Dr. J. Miller, USDA, Ames, IA.

[b]Model 651, American Optical Corp., Buffalo, NY.

[c]Dulbecco's formula, Flow Laboratories, Inc., McLean, VA.

[d]Performed by Dr. M. J. Schmerr, USDA, Ames, IA.

Other Species Examined

The DRU maintained between six and eight sheep and goats during the 27-month study. These animals were kept in a small pasture, separated from any cattle under study by at least two fences. Tests for presence of antibodies to BLV were negative for all sheep and goats in the fall of 1980 and 1981.

Several cats also inhabited the DRU during the study period. Three of the tamer cats were bled in October 1981, and no detectable BLV antibodies were found.

Diseases or Conditions Observed

During the 27-month study, lymphosarcoma was diagnosed in two cows following postmortem examination. Both were Jerseys, one six years old and the other five years old.

Although no health records were kept, nearly all neonatal calves experienced at least one episode of diarrhea and bronchopneumonia, and several died. According to clinical and pathological reports, a common cause of death, particularly prior to December 1979, was septicemia resulting from omphalophlebitis and/or hypogammaglobulinemia. Chronic dysentery was prevalent among calves two to six months of age. This was probably due, at least in part, to coccidia and trichostrongyles, as suggested by oocysts and eggs found on fecal flotation. Many calves in this age group, particularly bull calves in nutrition trials, were

quite cachectic. Heifers older than six months appeared less affected.

Data Collection and Computer Programs

Data were transcribed onto computer sheets from which IBM cards were punched. Files were maintained on tape at the Northeast Regional Data Center (NERDC), University of Florida, Gainesville, Florida. Computer systems available through NERDC for statistical analysis were the Statistical Analysis System (SAS) version 79.5,[a] Biomedical Computer Programs (BMDP),[b] and the McGill University System for Interacting Computing (MUSIC) version 4.1, 1978.

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[a]SAS User's Guide, SAS Institute Inc., Raleigh, NC.

[b]BMDP, P-series, University of California Press, Berkeley, 1979.

CHAPTER IV
IN UTERO TRANSMISSION OF BOVINE LEUKEMIA VIRUS

Introduction

Since BLV is currently considered an exogenous virus (Callahan et al., 1976; Kettmann et al., 1976, 1978a, 1978b, 1979a, 1979b; Kukaine et al., 1979) and there is no evidence for natural transmission of BLV via semen (Baumgartener et al., 1978; Miller and Van Der Maaten, 1979; Ressang et al., 1980), prenatal infection is most likely a consequence of in utero transmission. Suggested rates for in utero transmission of BLV infection have ranged from 3% to 25% (Ferrer et al., 1976, 1977a, 1977b; Piper et al., 1979). Highest rates were observed, however, in an inbred herd in which selection for bovine leukemia had been practiced (Piper et al., 1979). The BLV status of the dams was not stated in some reports (Ferrer et al., 1976, 1977a, 1977b); therefore, rates could have been higher had they been stated correctly in terms of calves born to infected cows.

It is not known what factors, if any, predispose a fetus to infection with BLV. Stage of gestation in which the dam was infected has not been associted with fetal infection (Van Der Maaten et al., 1981b). If predisposing

factors are present, calves and/or dams with the related characteristic could be segregated, thus reducing postnatal transmission. Otherwise, efforts could be focused on other aspects of transmission control.

The purpose of this chapter is to describe observed rates of natural precolostral antibodies to BLV in a large sample of random-bred calves and to examine for associations between those rates and certain characteristics of the calves and their dams.

Materials and Methods

Data Analyzed

Calves examined were those born to BLV-infected dams and bled precolostrally. A BLV-infected dam was defined as one which had BLV antibodies up to one month postpartum. Calves were divided into two groups: (1) those postcolostrally positive for BLV antibodies and followed for four months or more, or postcolostrally negative for BLV antibodies and followed for one month or more, and (2) those with postcolostral BLV antibodies and not followed for four months. A positive precolostral sample was considered invalid, if, after following the calf for four months or more, there was no evidence for BLV infection. Evidence for BLV infection was based on a rising antibody titer during the first six months of age, on observation of titers outside the 95% prediction level of decay of BLV colostral

antibodies (CHAPTER V), or on persistence of BLV antibodies beyond six months of age.

Analysis

Rates of precolostral antibodies in both groups of calves were examined for associations with breed and sex of calf. Analyses were performed using the FUNCAT program, a procedure for analysis of categorical data (Grizzle et al., 1969) offered by SAS. The model was presence (or absence) of precolostral BLV antibodies = breed + sex + breed x sex. The response function was the difference between the proportion of calves with and the proportion without precolostral antibodies to BLV, or, as indicated by SAS, 1 -1.

Differences in age and parity distributions for dams of calves with precolostral antibodies and dams of calves without precolostral antibodies were examined using the NPAR1WAY program of SAS, a two-sample Wilcoxon Rank Sums test for nonnormal distributions. Fisher's exact test, from the STATPAK subsystem of MUSIC, was used to calculate the probability of association between the stage of gestation of seroconversion to BLV by the dam and occurrence of precolostral antibodies in calves followed four months or more.

Results

Of 346 calves born during the period July 1, 1979, through September 30, 1980, 280 calves were bled

precolostrally and, of those, 223 were from BLV-infected dams. Because many calves were sold or were not followed for more than a few months, only 125 calves were born from BLV-positive cows, bled precolostrally, and followed for four months or more (Table IV-1). Eight (6.4%) of these calves had precolostral antibodies and antibodies persisting beyond six months of age (Table IV-1, IV-2). In the group of 223 calves, 18 (8.1%) had detectable precolostral antibodies (Table IV-1).

Precolostral antibodies were detected with similar frequency in Jersey and Holstein calves, both in the followed group ($p = 0.66$) and for all calves ($p = 0.80$) (Table IV-3). When all calves were examined, males showed a significantly higher rate of precolostral antibodies than did heifers ($p = 0.04$). There was no such association in the 125 calves followed ($p = 0.11$) (Table IV-3). No significant interactions between breed and sex were detected for either the group followed ($p = 0.91$) or for all calves ($p = 0.64$) (Table IV-3).

No statistical differences were detected between distributions of age ($p = 0.86$) or parity ($p = 0.83$) for dams having calves with precolostral antibodies and for dams having calves without detectable precolostral antibodies (Table IV-4). Complete serologic records were available for 76 cows seroconverting before the second month of gestation and for 14 cows seroconverting after the second month. Those seroconverting after the first trimester of

gestation showed no more tendency to have a calf with precolostral antibodies to BLV than did cows seroconverting before the first trimester (exact $p = 0.50$) (Table IV-5).

Discussion

The ruminant fetus may be particularly susceptible to infection because (1) syndesmochorial placentation does not allow transfer of maternal antibodies to the fetus; (2) the fetal immune system is not fully functional; and (3) microbial activity can take place in fetal cells (Osburn, 1981). Since placentation restricts passage of antibodies to the fetus, presence of fetal immunoglobulins is indicative of infection by, or exposure to, an antigen or of placental leakage of globulins (Brambell, 1970; Husband et al., 1972). Although no estimates are available for frequency of placental leaks, such events are believed to be rare (Brambell, 1970). This has been confirmed by several studies of *in utero* infection based on fetal infection and fetal or precolostral serology (Braun et al., 1973; Dunne et al., 1973; Fennestad and Borg-Petersen, 1962; Gibson and Zemjanis, 1973; Horner et al., 1973; Kniazeff et al., 1967; Osburn and Hoskins, 1971; Osburn et al., 1974; Van Der Maaten et al., 1981b).

The main difficulty when studying *in utero* infection as it occurs naturally in a population, using serologic detection, is one of logistics. Efforts should be made to sample the calf as soon after birth as possible and before

it nurses. For this reason, cows were fitted with udder bags a week or two before their due-date. However, prior to December 1979, research personnel were lax about udder bag procedures and precolostral sampling. As a result, some calves could have sucked BLV-positive cows without udder bags. Precolostral samples could be partially verified, however, by following calves to determine if they became serologically negative. For purposes of analysis, two groups of calves were described based on confidence in precolostral samples. A follow-up time of four months was selected because 80% of calves consuming colostrum containing BLV antibodies were negative by 120 days of age (CHAPTER V), and prediction of infected calves, therefore, could be made with confidence.

Radioimmunoassay using gp-51 antigen has been shown to be a highly sensitive test of BLV antibodies and may detect infected animals up to 10 days sooner than AGID (Miller et al., 1981). After screening with AGID, negative sera were retested by RIA to identify calves infected in late gestation. Additional calves identified as precolostrally seropositive by RIA may have consumed colostrum, as previously mentioned. More confidence, therefore, should be placed on the precolostral antibody rate of 6.4% than on that of 8.1% because of these logistical problems.

Since reported <u>in utero</u> infection rates of 10% or more may have counted calves born to negative dams, those figures represent minimal rates for calves born to infected

dams. In addition, syncytium induction assay, virus neutralization, and/or immunofluorescence assay were used in those studies to detect BLV or BLV antibodies. These tests may be less sensitive than RIA with gp-51 for detection of BLV infection (Miller et al., 1981; Van Der Maaten and Miller, 1977), and, therefore, in utero infection may have been underestimated further.

The most likely explanation for the discrepancy between rates observed in this study and those reported in other studies rests in innate features of the populations examined. Higher rates (e.g., 14%, 18%, and 25%) were observed in a Jersey herd in which animals had been inbred and selected for leukemia (Piper et al., 1979). Such high rates may suggest a weakening of placental integrity due to inbreeding allowing for passage of maternal blood into the fetus. In any case, it is unlikely that the higher rates are representative of in utero infection in a typical dairy herd.

A rate of 3% was observed in 37 calves from a multiple-case herd (Ferrer et al., 1976). Again the BLV status of dams was not reported, and, therefore, rates in calves from infected cows were possibly higher. Furthermore, caution should be taken in considering a rate based on only one infected calf from a small sample of 37 calves.

Results of this study indicated that the risk of BLV transmission did not differ between older, multiparous cows and younger or primiparous cows. This suggests that

transmission is not a consequence of loss of placental integrity associated with increasing physiologic age or pregnancies. In addition, stage of gestation at maternal infection did not appear to influence fetal infection rates, in accordance with observations from experimental studies (Van Der Maaten et al., 1981b).

Reports on studies of breed effects on BLV infection rates indicate that many breeds are susceptible (Burridge et al., 1981; Marin et al., 1978). One report concluded that higher infection rates occurred in Jerseys (Burridge et al., 1981), but these differences could have been attributable to high rates in particular age cohorts (Huber et al., 1981; Wilesmith et al., 1980). Furthermore, no breed effects were found in a previous study at the DRU (Burridge et al., 1979).

Although fetal sex showed some statistical association with precolostral antibodies when both groups were combined, this possibly resulted from less fastidious postnatal sampling of bull calves. Colostrum may have been consumed by more bulls than heifers prior to sampling because less attention is usually paid to postnatal care of bulls.

From a practical standpoint, knowledge of dam age or parity probably would not assist in control or prediction of <u>in utero</u> BLV infection. Economic justification for efforts to control <u>in utero</u> infection seems unlikely with a rate as low as 6.4%. Even though control may not be

possible, awareness of *in utero* infection is essential in planning postnatal management of BLV transmission.

Table IV-1. Summary of results of precolostral sampling for antibodies to bovine leukemia virus (BLV) between July 1, 1979, and September 30, 1980, at the University of Florida Dairy Research Unit, Hague, Florida.

	Holstein		Jersey		
Number of calves	Females	Males	Females	Males	Total
Born	136	112	47	51	346
Bled precolostrally	102	97	42	39	280
Born to BLV-infected dam	74	79	36	34	223
Followed for four months or more	67	13	35	10	125
Precolostral BLV antibodies (%)*	2(3.0)	2(15.4)	2(5.7	2(20)	8(6.4)
Not followed for four months	7	66	1	24	98
Precolostral BLV antibodies (%)†	0	8(12.1)	0	2(8.3)	10(10.2)
Total precolostral BLV antibodies (%)‡	2(2.7)	10(12.7)	2(5.6)	4(11.8)	18(8.1)

*Percentage based on number of animals followed for four months or more.

†Percentage based on number of calves followed less than four months.

‡Percentage based on all animals bled precolostrally and born from BLV-positive dams.

Table IV-2. Calves followed for six months or more which had precolostral and persisting antibodies to bovine leukemia virus (BLV).

Calf number	Dam parity	Dam age (months)	Breed	Sex
H875*	2	37	Holstein	F
H955*	3	59	Holstein	F
H1002B*	1	24	Holstein	M
H1038B*	2	37	Holstein	M
J166*	3	56	Jersey	F
J171†	2	35	Jersey	F
J194B†	1	23	Jersey	M
J200B*	5	73	Jersey	M

*Presence of antibodies determined by agar-gel immunodiffusion using glycoprotein antigen.

†Antibodies were detectable only by radioimmunoassay using glycoprotein antigen.

Table IV-3. Associations of sex and breed of calf with presence of precolostral antibodies to bovine leukemia virus (BLV).

Group	n	df	Chi Square	Probability
Calves followed four months or more	125			
Intercept		1	86.87*	0.0001
Sex		1	2.54	0.1107
Breed		1	0.19	0.6606
Sex by breed		1	0.01	0.9102
All calves	223			
Intercept		1	446.83	0.0001
Sex		1	4.17	0.0411
Breed		1	0.06	0.8045
Sex by breed		1	0.22	0.6360

*Analyses performed using the FUNCAT procedure for categorical data analysis, SAS User's Guide, 1979.

Table IV-4. Results of Wilcoxon Rank Sums test for association of dam age and parity with presence of precolostral antibodies to bovine leukemia virus (BLV) in offspring.

Precolostral BLV antibodies[+]	Number of offspring[±]	Dam age[*] (months)				Dam parity			
		Sum of ranks[§]	Expected under HO	Mean rank	p>|z|	Sum of ranks	Expected under HO	Mean rank	p>|z|
Present	8	482	504	60.25		486	504	60.75	
					.8283				.8599
Absent	117	7393	7371	63.19		7389	7371	63.15	

[*]All dams were positive for BLV antibodies.

[+]Determined by agar-gel immunodiffusion and radioimmunoassay using glycoprotein antigen.

[±]Only offspring followed for four months or more were included in the analysis.

[§]Midranks were used for ties.

Table IV-5. Frequency of precolostral antibodies to bovine leukemia virus (BLV) in calves born to cows with antibodies to BLV prior to the second month of gestation and in calves born to cows seroconverting sometime between the second month of gestation and parturition.

Antibodies to BLV in precolostral serum*	Number of calves		
	Stage of gestation in which the dam seroconverted		
	Before second month	After second month	Total
Present	4+	0	4
Absent	72	14	86
Total	76	14	90

*Presence of antibodies determined by agar-gel immunodiffusion and radioimmunoassay.

+Fisher's exact probability = 0.502.

CHAPTER V
DECAY OF COLOSTRAL ANTIBODIES TO BOVINE LEUKEMIA VIRUS

Introduction

Before postnatal transmission of BLV can be studied, criteria for serologic detection of BLV infection must be established. This is straightforward for an animal which seroconverts after a period of seronegativity. However, during the period when colostral antibodies are detectable, it is difficult to discriminate between infected and non-infected calves strictly by the presence or absence of antibodies. In order to more accurately define time or place of infection, it would be helpful to detect infected calves during the period that colostral antibodies are detectable.

This chapter will describe the decay of colostral BLV antibodies in noninfected and infected calves and estimate normal limits of decay in noninfected calves. It is shown how these normal limits can be used to detect BLV-infected calves during the first six months of life.

Materials and Methods

Data Analyzed

Titers were analyzed from calves which had colostral antibodies on at least three observations, followed by no

serologic reaction for at least two months after the last colostral antibody titer. These calves were assumed not to be infected with BLV during the period when colostral antibodies were detectable because, had the virus been present, an active antibody response probably would have been observed within two months after the last detectable colostral titer (Mammerickx et al., 1980; Van Der Maaten and Miller, 1978b, 1978c). Furthermore, apparently to date, there is no evidence that the presence of BLV appreciably alters the rate of decay of colostral antibodies.

Titers also were analyzed from 14 calves which either had precolostral antibodies that persisted throughout the study or had antibodies persisting through eight months of follow-up. These calves were assumed to have been infected during at least part of the colostral-antibody period. Only calves with titers which remained the same or declined from previous levels were included in regression analysis. All titers from infected calves were included in estimates of ages at which BLV infection could first be serologically detected.

Analysis

Titers were analyzed using an estimated weighted regression procedure (Swamy, 1971). All computations were performed using SAS. A linear regression predicting the $\log_{10}$ of the inverse of BLV antibody titer at various ages was determined by pooling intercepts and slopes from

individual calf least squares regressions. Each vector of regression coefficients was weighted by the inverse of its estimated covariance matrix. The mathematical model was

$$Y = a + bX + u$$

where Y (dependent variable) was $\log_{10}$ of the inverse of the end-point titer, a was the pooled Y intercept, b was the pooled rate of decay of $\log_{10}$ inverse titer, X (independent variable) was age in days when the titer was observed, and u was random error. Prediction bands (normal limits) were calculated for the 90%, 95%, and 99% confidence levels. A zero titer level was set at $\log_{10}$ (0.5) = -0.3. Half-life of antibodies was estimated by

$$\frac{\sum_{i=1}^{n} \frac{\log_{10}(10^{a_i}) - a_i}{2}}{\frac{b_i}{n}}$$

where a_i and b_i were the Y intercept and slope of the estimated regression line for the i^{th} calf and n = 130 calves.

A randomly selected set of 61 calves was used to obtain preliminary estimates of a and b and normal limits. The validity of these estimated normal limits of BLV antibody decay was tested using the remaining 69 calves as a validation group. Since approximately 91%, 94%, and 98% of the observations in the validation group fell within the 90%, 95%, and 99% normal limits established by the first

set of calves, the two sets were combined and a final prediction model was derived based on all 130 calves.

Regression analysis was repeated for 14 infected calves which had declining titers for at least three observations beginning at two days of age. Tests of hypotheses of equal intercepts and slopes of colostral decay were made using large approximate t statistics (Swamy, 1971, p. 129).

Results

For the 130 noninfected calves, the number of positive antibody titers per calf ranged from three to eight. One of these calves had an early titer as high as 1:256. Duration of colostral-BLV antibody titers ranged from 51 to 187 days with 50% of the calves serologically negative by 95 days of age (Fig. V-1).

The regression equation for all 130 calves was $Y = 1.29 - 0.012X$. The mean half-life of BLV antibodies was estimated to be 27.1 days ± 1.2 days. The prediction line crossed the zero titer level at 136 days of age, while the 90%, 95%, and 99% upper prediction bands crossed at 168, 178, and 200 days of age, respectively (Fig. V-2).

Serologic diagnosis of BLV-infected calves was made between 2 and 180 days of age using the 90% limit and between 8 and 206 days of age using the 99% limit (Table V-1).

The regression line of BLV colostral antibody decay for 14 infected calves was $Y = 1.43 - 0.012X$ (Fig. V-3).

No differences were found between noninfected calves and infected calves for slopes ($p = 0.45$) or intercepts ($p = 0.43$).

Discussion

Examination of individual calf regressions revealed little deviation from linearity when titers were expressed on the $\log_{10}$ scale. The decision to set the level of a negative titer at $\log_{10}(0.5)$ was based on the two-fold dilution scheme. Extrapolation below a titer of 1:1 can be made with confidence because there is no reason to suspect appreciable deviation from exponential decay below the level detectable by AGID.

Some assumptions for estimated weighted regression analysis (Swamy, 1971) could not be perfectly met in this application. The distribution of $\log_{10}$ of inverse titers, for example, cannot be assumed to be normal, particularly as calves age, because titer values truncate at zero. Also, the method of titer measurement results in a discrete dependent variable which would not have an exact normal distribution. It is likely that with a sample of 130 calves violations of these assumptions are not critical. Nevertheless, 69 calves were assigned randomly to a validation set to test validity of the 90%, 95%, and 99% normal limits. Since results showed little deviation from expectations, it can be assumed that these limits were valid. Estimated weighted least squares analysis was performed instead of ordinary least squares regression because the assumption of

independence of deviations from overall regression does not hold when repeated measures are made on each animal (Swamy, 1971).

It is well known that IgG is the major immune globulin acquired from colostrum by the neonatal calf (Pierce and Feinstein, 1965; Porter, 1972). These colostrum-derived immunoglobulins originate from the maternal circulation where they transfer to colostrum several weeks prior to parturition (Brandon et al., 1971; Logan et al., 1973; Smith, 1971; Sullivan et al., 1969). Since AGID using BLV gp-51 antigen measures IgG_1 exclusively (Matthaeus et al., 1978), decay of colostral BLV antibodies is essentially the decay of IgG_1. The half-life of IgG_1 in the bovine may vary from 18 to 22.7 days (Brar et al., 1978; Dixon et al., 1952; Logan et al., 1973; MacDougal and Mulligan, 1969; Smith, 1971). The longer half-life of 27.1 ± 1.2 days found in this study may have several explanations. Use of larger and older animals in at least one study may account for a shorter half-life, since younger and smaller animals may metabolize IgG_1 at a slower rate (Dixon et al., 1952). Furthermore, the rate of decay of IgG_1 may be dependent on the incidence of diarrhea in calves studied (MacDougal and Mulligan, 1969).

The 187-day duration of colostral antibody titers to BLV found in this study was similar to that for infectious bovine rhinotracheitis (Brar et al., 1978) and bovine viral diarrhea (Brar et al., 1978; Kahrs et al., 1966; Kendrick

and Franti, 1974). In another study of 21 calves, colostral antibodies to BLV were detected for only 154 days (Fischer and Keyserlingk-Eberius (1980). Since AGID has not been standardized, a discrepancy of this magnitude is not surprising. A lower rate of diarrhea or consumption of a higher quality or quantity of colostrum also could explain the longer duration of antibodies found in our study.

The age at which infected calves could be detected was variable within a given level of normal limits. This is not unexpected because the critical level at which viral replication is initiated also is variable and dependent on both neutralizing antibody and virus concentration (Driscoll et al., 1977; Straub, 1978b). At the 90% prediction level, infected calves were identified as early as 2 days and as late as 180 days of age. At the 99% level, calves were identified later, from 8 to 206 days of age, but fewer noninfected calves were misidentified. The sensitivity of this method varies inversely, whereas specificity varies directly, with the level of precision (prediction).

Serologic detection of BLV-infected calves during the period of colostral antibody decay could be used in eradication or control programs where it would be feasible to serially dilute serum from calves less than six months of age. Because typical levels of passive antibodies vary from region to region and herd to herd (Naylor, 1979), a decay curve might be defined for noninfected calves which is characteristic of a herd or region. Since at least three

positive observations are required per animal to estimate error variance, calves could be bled at weekly intervals for the first month and at monthly intervals thereafter.

This technique of defining upper limits of colostral antibodies also could be used in planning strategies for herd immunization (Kahrs et al., 1966; Kendrick and Franti, 1974). Calves would be vaccinated at the earliest age that passive antibodies would not interfere with immunization. Other factors, such as herd disease prevalence, antigen dose and type, and general health conditions, also should be considered (Brar et al., 1978; Kahrs et al., 1966; Kendrick and Franti, 1974; Uhr and Bauman, 1961).

Colostral antibodies decayed at the same rate from the same level in infected calves as in noninfected calves. This validated the assumption that presence of BLV did not alter BLV colostral antibody decay. Use of calves with no evidence of BLV infection for at least two months beyond the last colostral titer, therefore, should provide valid estimates of BLV colostral antibody decay.

Table V-1. Ages at which bovine leukemia virus (BLV) antibody titers from BLV-infected calves were above normal limits of colostral titers of noninfected calves.

Infected calf number	Age (days) at which $\log_{10}$ 1/titer* was first above the normal limits of		
	90%	95%	99%
J166†	2	2	178
J171†	153	153	153
J183‡	151	151	151
J194B†	169	169	169
J200B†	38	73	206
H875†	180	180	180
H888§	154	154	154
H913‡	155	155	155
H938§	112	112	167
H949§	2	2	8
H955†	160	160	160
H972§	137	137	163
H973§	67	67	102
H1002B†	31	31	31
H1038B†	27	27	144
H1079B§	167	167	167
MEAN	106.56	108.75	143.00
SEM	16.54	16.07	13.22

*Antibodies measured by agar-gel immunodiffusion using glycoprotein antigen.

†Calves with precolostral antibodies to BLV.

‡Calves with no detectable precolostral antibodies to BLV.

§Calves which were not sampled precolostrally.

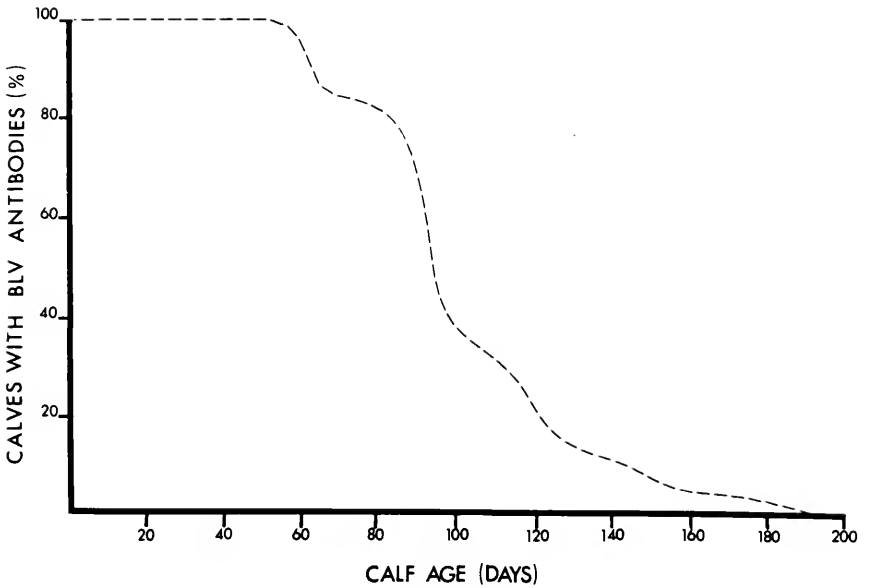


Fig. V-1. Percentage of 130 calves with colostral antibodies to bovine leukemia virus (BLV) as a function of age.

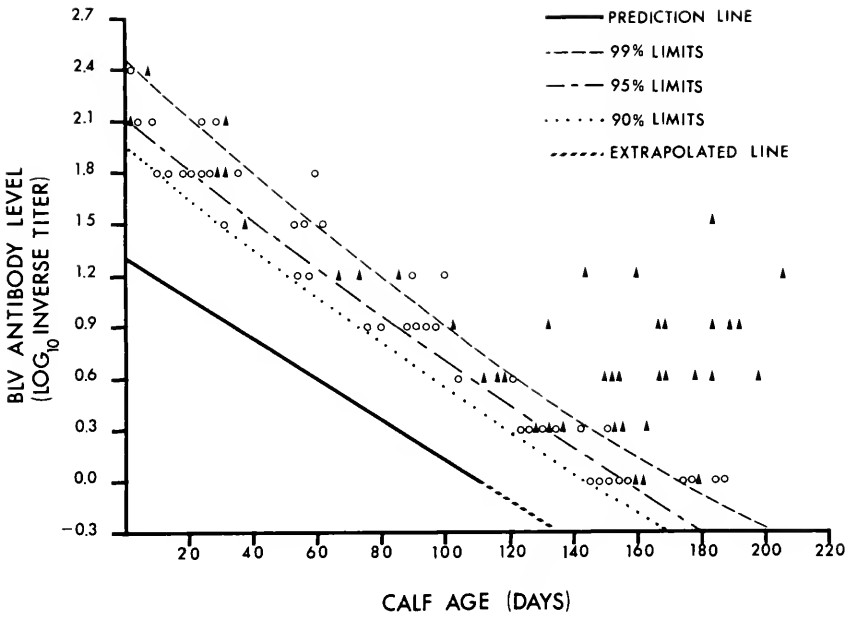


Fig. V-2. Prediction line of the decay of colostral antibodies to bovine leukemia virus (BLV) in 130 calves with no evidence of infection with BLV. Upper normal limits of the prediction line are indicated for the 90%, 95%, and 99% confidence levels; o = values of $\log_{10}$ 1/titer falling above the 90% normal limit from calves not infected with BLV; ▲ = values of $\log_{10}$ 1/titer from calves infected with BLV in utero or sometime prior to six months of age.

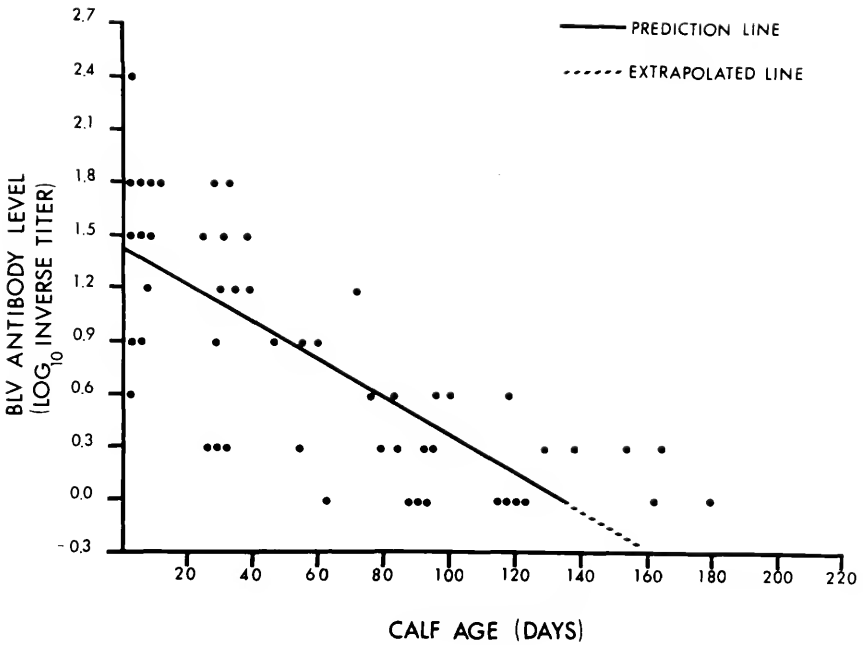


Fig. V-3. Prediction line of the decay of colostral antibodies to bovine leukemia virus (BLV) in 14 calves infected with BLV in utero or sometime prior to six months of age; ● = values of $\log_{10}$ 1/titer.

CHAPTER VI
AGE-SPECIFIC RATES OF DETECTION OF BOVINE LEUKEMIA VIRUS INFECTION

Introduction

Several factors have been examined individually for association with prevalence rates of BLV infection in previous studies. Rates have been reported to be unrelated to BLV-status of the dam (Hofirek, 1980; Olson et al., 1978; Valikhov, 1978), consumption of colostrum from BLV-infected cows (Ferrer et al., 1976; Ferrer and Piper, 1978; Piper et al., 1975, 1979), or breed (Burridge et al., 1979). Other studies have suggested that an association exists between prevalence rates of BLV infection and breed (Burridge et al., 1981), BLV status of the dam (Baumgartener et al., 1978), type of colostrum consumed (Seger and Morgan, 1977), and age (Burridge et al., 1979, 1981; Chander et al., 1978; Evermann et al., 1980; Ferrer et al., 1976; Hofirek, 1980; Huber et al., 1981; Mammerickx et al., 1978a, 1978b; Marin et al., 1978; Olson et al., 1973; Parfanovich et al., 1978; Piper et al., 1979). Because age is associated with BLV prevalence rates, it is important to examine for the age-specific effects of factors on rates of infection.

The objective of this chapter is to describe age-specific rates of detection of BLV infection and to test

for differences between rates for cattle identified by various maternal and management features.

Materials and Methods

Cattle Studied and Criteria for Detection of BLV Infection

Cattle studied were those described in CHAPTER III. Calves known to be infected *in utero* (CHAPTER IV) were excluded from examination of postnatal infection. Detection of BLV infection was made when BLV-gp antibodies first appeared following a period of seronegativity. In calves showing evidence of colostral BLV antibodies, e.g., a declining titer during the first six months of life, the time at detection of BLV infection was determined from the model of colostral antibody decay (CHAPTER V). A calf was detected as infected when titers first began to increase or when a titer fell outside the 95% normal limits of colostral antibody decay, whichever occurred first. Because the precise moment of infection could not be measured, the date of detection was used to approximate this time, assuming a uniform lag period from infection to detection in all cattle.

A BLV-infected dam was defined as one which had antibodies to BLV during gestation or within one month following parturition. A calf was judged to have consumed colostrum from a BLV-infected cow if the calf had detectable antibodies to BLV in the first postcolostral sample and was

precolostrally negative or if it had postcolostral antibody titers that declined with age.

Analysis

Data were analyzed using survival regression techniques (Cox, 1972; Taulbee, 1979) available in the PHGLM program of SAS. Life-table calculations and graphics were performed using the statistical program P1L of BMDP. Failure time was defined as the age in days when an animal was detected as infected with BLV. Censored time was the age when an animal died, left the herd, calved, or when follow-up was terminated on September 30, 1981, whichever occurred first.

The number of cattle at risk of being detected as infected in an age interval t_i, defined for graphical purposes as 30 days, was

$$r_i = n_i - \frac{c_i}{2} ,$$

where n_i was the number of animals entering the interval and c_i the number censored in the interval. Conditional probability of detection in an interval (given that detection did not occur in the previous interval) was

$$q_i = \frac{d_i}{r_i} ,$$

where d_i was the number detected as infected in the interval. It was assumed that risk of detection remained constant during an interval. The conditional probability of an

animal not being detected (surviving) in the i^{th} interval was

$$p_i = 1 - q_i .$$

The cumulative proportion remaining undetected (also known as cumulative survival or survival rate) to the beginning of the i^{th} interval was

$$P_i = p_{i-1}P_{i-1} ,$$

where $P_1 = 1$. Hazard rates (also called failure rates or force of morbidity) were estimated at interval midpoints and were defined as the quantity

$$\lambda_i = 2q_i/h_i(1+p_i) ,$$

where h_i was the width of the i^{th} interval. This rate was a linear interpolation estimate based on a life table representation of a survival curve.

Standard errors of cumulative survival functions and hazard rates were calculated as described in the PlL program of BMDP for all animals born between July 1979 and June 30, 1981.

Relationships between hazard rates and other variables were assessed using the model of the hazard at time t, as previously described (Cox, 1972) because it adjusts for censoring in the data. This model is

$$\lambda(t,z_i) = \exp(z_i\beta)\lambda(t) ,$$

where β is a vector of unknown parameters and z is the variable under study. The variable z can take on indicator values (e.g., 1 = positive dam and 0 = negative dam) or continuous values as in dam age. Implicit in this model is the assumption that hazard rates for individuals with different values of the covariate are in constant ratio over time, regardless of the underlying hazard rate (Taulbee, 1979). An obvious violation of the proportional hazards assumption occurs when survival curves cross. This often is the case with actual data, and it is possible to examine changes in hazard rates with age using an alternative function described by Taulbee in which a new variable, zt, is defined. This allows the ratio of hazard functions to differ and permits examination for age-by-factor interactions. Conclusions based on this test, however, must be extremely tentative, since the differences detected as significant are unknown.

Correlation coefficients were calculated using the CORR program of SAS to describe relationships between the various factors investigated. To examine effects of factor interactions on survival, correlation coefficients were computed from the variance-covariance matrix of parameter estimates when all factors were included in the survival model. High negative correlations between regression parameters for two factors were interpreted as a reduction in the effectiveness of one factor in explaining survival when the role of the other factor on survival became more

important. High positive correlations identified factors which were jointly effective in explaining survival.

Factors examined were dam age (months), dam parity (0 = primiparous, 1 = multiparous), dam BLV status when the animal under study was born (1 = positive, 0 = negative), type of colostrum consumed (1 = from a BLV-infected cow, 0 = not from a BLV-infected cow), and breed (1 = Hostein, 0 = Jersey).

Results

A total of 473 live calves entered the survival analysis at birth. Of these, nine were detected as infected *in utero* (CHAPTER IV) and 54 infected postnatally. Two animals were followed through 780 days of age (Table VI-1). Estimates of prevalence rates of infection increased from 2.3% at birth to 63% at 27 months of age (Fig. VI-1). Survival rates (Fig. VI-1), hazard rates (Fig. VI-2), and proportion of animals detected as infected for various age groups (Fig. VI-3) demonstrated four possible age-related stages of detection. The first stage, prenatal infection, has been characterized previously (CHAPTER IV). A second stage appeared as increased hazard rates during the first six months of life. This was followed by a 10-month period of sporadic detection. The fourth stage, beginning at 16 months of age, was characterized by sharply increasing hazard rates of detection through 27 months of age.

Results of statistical analyses using Cox's procedure are presented in Table VI-2. No differences were found in hazard rates when variables were examined separately without the age covariate. However, significant age interactions were suggested for all factors studied after inclusion of zt and use of the test by Taulbee (Table VI-2). Graphically, factor-by-age interactions were indicated for calves consuming BLV-positive colostrum (Fig. VI-4), for dam status (Fig. VI-5), for breed (Fig. VI-6) and for dam parity (Fig. VI-7). Lower rates of detection of BLV infection were observed in young calves from infected dams and in calves consuming colostrum from infected cows. Higher rates of detection were observed in older Jerseys than in Holsteins of the same age. Calves born to primiparous dams appeared to have lower rates of detection through 18 months of age, after which they began to experience higher rates of detection until 27 months of age when rates were similar.

Significantly high factor correlations were found between type of colostrum consumed and dam status ($p = 0.0001$) and between dam age and parity ($p = 0.0001$), and low, but significant, correlations were found between dam parity and dam status ($p = 0.0001$), between colostrum and parity ($p = 0.001$), and between colostrum and dam age ($p = 0.001$) (Table VI-3). High, negative correlation coefficients of estimated parameters of survival were found only for dam age and parity ($r = -0.97$) and for colostrum and dam status ($r = -0.86$) (Table VI-4).

Discussion

Throughout this study increasing or persisting antibody titers to BLV, as measured by AGID-gp, were interpreted as evidence for BLV infection. Because this method indirectly tests for presence of virus by an immune response, some assumptions regarding sensitivity and specificity of the diagnostic criteria should be made.

The BLV-gp antigen appears to be unique, since no cross reactivity with proteins of other oncoviruses or retroviruses has been demonstrated (Ferrer, 1972; Ferrer et al., 1975; Kaaden et al., 1977; McDonald and Ferrer, 1976; McDonald et al., 1976). A positive serologic response to BLV-gp, therefore, can be considered specific for BLV.

Comparisons of several serologic tests have demonstrated that AGID-gp is a relatively sensitive serologic test (Burny et al., 1980). These results are corroborated by experimental studies in which a serologic response following BLV inoculation was consistent (Miller et al., 1972; Schmidt et al., 1976; Van Der Maaten and Miller, 1978b; Van Der Maaten et al., 1981a) and persistent (Miller and Van Der Maaten, 1976b). Similarly, BLV has been readily detected from seropositive but not seronegative animals following natural infection (Ferrer et al., 1976, 1977a, 1977b; Olson et al., 1973; Piper et al., 1979).

There is no evidence for recovery from BLV infection. On the contrary, seropositive cattle older than six months

appear to maintain detectable antibodies throughout life (Chander et al., 1978; Kaaden et al., 1978; Tabel et al., 1976). In a study to examine vaccine efficacy, inactivated BLV produced only a transient serologic response, and virus was not isolated (Miller and Van Der Maaten, 1978b). In that study two doses of killed virus were required to induce a serologic response. This suggests that persistent antibody titers only result from active, persistent infection and that any transient serologic response resulting from exposure to a killed virus is rare.

Until recently, it could not be stated that absence of either virus or antibody implied absence of specific BLV sequences in host cell genomes. Studies now suggest that such sequences are not to be found in cattle without BLV or specific BLV antibodies (Callahan et al., 1976; Deschamps et al., 1981; Kettmann et al., 1976).

A final demonstration of the sensitive nature of AGID using gp-51 lies in results of BLV-eradication programs based upon mass screening with this test. Results of these campaigns indicated that the virus was efficiently removed from a herd by test-and-slaughter methods (Mammerickx et al., 1978a; Schmidt et al., 1978, Straub, 1978b). If AGID were not an efficient discriminator of infected and noninfected cattle, it is unlikely that eradication efforts would have met with such rapid success. Use of AGID-gp to detect BLV infection, therefore, is justified, particularly in a design

in which animals can be followed for several months and persisting titers can be observed.

Survival analysis and life-table methods are well established techniques in epidemiology and actuarial science (Gross and Clark, 1975; Lilienfield, 1976). Recently these methods have seen increased application in veterinary epidemiology (Cobo-Abreu et al., 1979; Hird et al., 1975; Schwabe et al., 1977). Survival analyses are especially appealing in prospective studies where animals lost to follow-up can be considered in computations up to the time of censoring, assuming failure rates similar in censored and uncensored animals.

Data presented in this study are particularly suitable for examination by survival methods for two reasons. Firstly, recovery from BLV infection is unknown and animals can be defined as truly failed once infection is detected. This is analogous to death in traditional applications. Secondly, since these methods incorporate the dimension of time, either by comparing distributions over time or by using time as a dependent variable, they are appropriate in studies of BLV infection because prevalence rates are known to be associated with age (Burridge et al., 1979, 1981; Chander et al., 1978; Evermann et al., 1980; Ferrer et al., 1976; Mammerickx et al., 1978a, 1978b; Marin et al., 1978; Olson et al., 1973; Piper et al., 1979).

Cox's model was used to test estimated parameters of a survival function where the underlying assumption was

that hazard rates were proportional (Cox, 1972; Taulbee, 1979). The model tends to force nonparallel curves to a parallel model. Thus, interaction, characterized by crossing or bellying curves, could not be examined. Inclusion of the zt variable removes dependence on proportionality and allows for tests of interactions with age (Taulbee, 1979).

Estimated age-specific prevalence rates were calculated as one minus the proportion remaining negative for BLV infection. The prevalence rate estimate for 18-month-old cattle of 25% was lower than that of 45% described from a cubic polynomial curve fitted to data collected from the same herd for the years of 1975 through 1977 (Burridge et al., 1979). Transmission rates of BLV infection, therefore, appeared to have declined over the past four to five years, at least in animals up to 18 months of age. This may suggest variations of prevalence rates in yearly birth cohorts, as found in other prospective studies (Huber et al., 1981; Wilesmith et al., 1980). In those studies, it was observed that cattle born in a given year experienced a different life-time prevalence rate than did animals born in some other year. Furthermore, the prevalence rate within a cohort did not change appreciably beyond two years of age. A similar situation may have occurred in this herd due to management of Holstein heifers. Prior to 1977, bred Holstein heifers were kept in the dry herd until shortly before calving. In the past few years, these heifers have been managed separately from the dry herd,

whenever pasture space permitted, in order to provide them with a better plane of nutrition. In this study Holstein heifers were exposed to the dry herd for a relatively short time, January through April 1981, which could have limited BLV transmission and resulted in the lower prevalence rates.

Inspection of the overall life table revealed heavy censoring during the first two to three months. This was due to sales of bull calves and to neonatal mortality.

Early postnatal detection of BLV infection, constituting the second detection phase, could have been represented by calves infected in utero and not sampled precolostrally or infected too late in gestation for antibodies to appear by birth, as previously suggested (Van Der Maaten et al., 1981b). Evidence that postnatal infection occurred during this period was demonstrated by detection of infection in calves born to noninfected dams.

The length of this period of detection would not correspond to the length of an underlying infection period because of the seroconversion lag time (CHAPTER VIII) and possible repression of viral expression by colostral antibodies. Inhibition of release of BLV by antibodies has been demonstrated in vitro (Driscoll et al., 1977) and also suggested in studies of colostral antibodies (Van Der Maaten et al., 1981a). Antigenic modulation or inhibition of P proteins (Sissons and Oldstone, 1980) may explain such a phenomenon. The process of modulation is believed to involve a binding of viral glycoprotein on the cell membrane

by circulating antibodies. Glycoprotein surface markers become masked from recognition by K cells or other cells invoking an antibody dependent cytotoxic response. A failure to recognize virus-bearing lymphocytes by the immune system renders them resistant to immune damage. If antibodies are removed, for instance through natural attrition of gammaglobulins, the lymphocyte would regain the ability to shed virus. Inhibition of P proteins may also be involved in repressing virus expression by inhibiting transcription.

A further delay in detection, beyond the two- to three-month seroconversion period and one-month sampling interval could have resulted from early sampling-design flaws. As much as a two-month delay could have occurred as a result of the three-month sampling interval used at the beginning of the study. It is likely, therefore, that infection represented by this phase of detection occurred in the first two to three months of life or even prenatally.

Several factors may have contributed to postnatal transmission in the first two to three months. Calves may have become infected at parturition when a calf born to an infected dam ingested maternal blood from uterine or vaginal tears (Van Der Maaten et al., 1981b). Rates of detection in calves from infected dams, at least during the first three months, however, were not different from rates in calves from noninfected dams. Such a similarity of rates may be an artifact due to delayed expression of the virus

by colostral antibodies. Consumption of colostrum with BLV antibodies, therefore, may complicate control efforts by masking early infection.

Type of calf management also may have influenced BLV transmission during the first few months of life. Most calves were placed inside the calf barn where they could have been exposed to infected calves in adjacent pens. Transmission may have resulted from ingestion or inhalation (Van Der Maaten and Miller, 1978c) of saliva (Ressang et al., 1980), urine (Gupta and Ferrer, 1980), blood (Mammerickx et al., 1980; Van Der Maaten et al., 1981a), or lymphocytes (Miller and Van Der Maaten, 1978b; Van Der Maaten and Miller, 1978b, 1978c, 1981a). Animals were also tattooed while in the barn, introducing the opportunity for iatrogenic transmission. Other calves occupied reasonably isolated pens outdoors, and they may not have experienced the same risks of infection as indoor calves. Risks for animals in various locations are examined further in CHAPTER VIII.

A third relatively quiescent phase of detection of BLV infection between 6 and 15 months of age coincided with movement of animals from densely occupied paddocks to fields and pastures (APPENDIX D, Fig. 7). It also was noted that vaccinations for other infectious diseases were given either shortly before or during this period. Iatrogenic transmission of BLV, therefore, does not appear to have been of major importance in transmission because rates of detection actually declined during this period. A more

detailed examination of detection rates and vaccination procedures is presented in CHAPTER VII.

A sharp increase in detection rates typified the fourth and last observed phase which began after 15 months of age. The only management interventions preceding the increased rates were insemination and movement of bred heifers to the dry herd. Infection as a result of close physical contact has been suggested as the major mode of BLV transmission (Dechambre et al., 1968; Maas-Inderwiesen et al., 1978; Straub, 1971, 1978a; Miller and Van Der Maaten, 1978a; Wilesmith et al., 1980). Other reports mentioned similar observations or seroconversions following exposure to infected animals (Ferrer et al., 1976; Piper et al., 1979). However, in our study not all bred heifers entered the dry herd, permitting a comparison of risks of two types of heifer management in CHAPTER VIII.

It is not likely that transmission resulted from insemination, since the exogenous nature of BLV excludes transmission via gametes. This has been confirmed by observational studies which did not find increased infection rates in animals sired by BLV-infected bulls (Baumgartener et al., 1978) and by experimental studies which failed to transmit BLV with semen from BLV-infected, but otherwise healthy, bulls (Miller and Van Der Maaten, 1979; Ressang et al., 1980). Furthermore, the time from breeding (13-16 months of age) to peak rates of detection (18-21 months of age) was longer than suggested by experimental or

natural transmission studies (Gentile and Rutili, 1978; Mammerickx et al., 1980; Straub, 1978b; Van Der Maaten and Miller, 1978a, 1978b). Unfortunately, an effect of insemination on transmission could not be examined further because BLV status of sires was unknown and analysis would have been confounded by the presence of only bred heifers in the dry herd.

In the examination for associations between hazard rates and dam age, dam parity, dam status, breed, and colostrum type using Cox's model, no significant main effects were observed. Results of Taulbee's alternative procedure, however, suggested significant departures from proportional hazards. The sizes of the differences detected by this alternative method are not known, and they may be too small to be of practical importance in the control of BLV infection. Conclusions regarding the practical significance of interactions, therefore, should be very tentative, but discussion can be based on inspection of survival curves. Graphical examination is meaningful because crossing of survival curves cannot occur without crossing of hazard curves, thus allowing for visual inspection of factor-by-age interaction.

Interactions between age and dam status and between age and colostrum have already been discussed and indicated that calves which consume colostrum from an infected cow or which were born to a seropositive dam were protected from detection but not necessarily infection, at an early age.

These two factors would be expected to have similar curves because of the high negative correlation of their estimated survival parameters. Although several factors were significantly correlated with each other, only dam parity and dam age, and dam status and colostrum were correlated when hazard rates of detection were considered.

An interaction of breed by age was suggested when old Jersey heifers were observed to have higher rates of detection than old Holstein heifers, but at young ages curves were similar. It has been reported that Jerseys have low levels of circulating gammaglobulins (Logan et al., 1981), but this would not necessarily explain increased susceptibility only in old Jersey heifers. A more reasonable explanation for lower survival rates in old Jersey heifers is that they remained in the dry herd until shortly before calving. Holstein heifers, on the other hand, were removed from the dry herd on about May 1, 1981, and Holsteins bred 45 days prior to that time were never in the dry herd. The breed-by-age interaction, therefore, was due most likely to longer exposure of Jerseys to older, infected cattle in the dry herd.

There has been no mention made in the literature of dam parity or dam age effects on subsequent BLV infection of progeny. Crossing of survival curves of animals from primiparous and multiparous dams observed in this study suggests an age-by-parity or age-by-dam age interaction. Whether the crossing of these curves represents expected

variation, especially with small numbers at older ages, or a real effect cannot be stated. No other explanation can be provided.

Table VI-1. Life table and survival analysis of detection of bovine leukemia virus infection.

Age Interval (days)	Number of animals				Proportion		Cumulative survival (S.E.)	Hazard (S.E.)
	Entered	Withdrawn	Detected	At-risk	Detected	Surviving		
0- 30	473	158	9	394.0	0.023	0.977	1.0 (0.0)	0.0008 (0.0003)
30- 60	306	29	1	291.5	0.003	0.997	0.9772 (0.0075)	0.0001 (0.0001)
60- 90	276	18	5	267.0	0.019	0.981	0.9738 (0.0082)	0.0006 (0.0003)
90-120	253	18	6	244.0	0.025	0.975	0.9556 (0.0114)	0.0008 (0.0003)
120-150	229	7	1	225.5	0.004	0.996	0.9321 (0.0146)	0.0001 (0.0001)
150-180	221	18	4	212.0	0.019	0.981	0.9279 (0.0151)	0.0006 (0.0003)
180-210	199	21	6	188.5	0.032	0.968	0.9104 (0.0172)	0.0011 (0.0004)
210-240	172	5	0	169.5	0.0	1.0	0.8815 (0.0203)	0.0 (0.0)
240-270	167	8	0	163.0	0.0	1.0	0.8815 (0.0203)	0.0 (0.0)
270-300	159	11	1	153.5	0.007	0.993	0.8815 (0.0203)	0.0002 (0.0002)
300-300	147	10	0	142.0	0.0	1.0	0.8757 (0.0210)	0.0 (0.0)
330-360	137	9	2	132.5	0.015	0.985	0.8757 (0.0210)	0.0005 (0.0004)

Table VI-1. Continued.

Age Interval (days)	Number of animals				Proportion		Cumulative survival (S.E.)	Hazard (S.E.)
	Entered	Withdrawn	Detected	At-risk	Detected	Surviving		
360-390	126	15	0	118.5	0.0	1.0	0.8625 (0.0226)	0.0 (0.0)
390-420	111	12	2	105.0	0.019	0.981	0.8625 (0.0226)	0.0006 (0.0005)
420-450	97	11	1	91.5	0.011	0.989	0.8461 (0.0250)	0.0004 (0.0004)
450-480	85	12	0	79.0	0.0	1.0	0.8368 (0.0264)	0.0 (0.0)
480-510	73	1	3	72.5	0.041	0.959	0.8368 (0.0264)	0.0014 (0.0008)
510-540	69	2	5	68.0	0.074	0.926	0.8022 (0.0320)	0.0025 (0.0011)
540-570	62	3	3	60.5	0.050	0.950	0.7432 (0.0390)	0.0017 (0.0010)
570-600	56	3	5	54.5	0.092	0.908	0.7064 (0.0425)	0.0032 (0.0014)
600-630	48	5	3	45.5	0.066	0.934	0.6415 (0.0475)	0.0023 (0.0013)
630-660	40	5	2	37.5	0.053	0.947	0.5992 (0.0502)	0.0018 (0.0013)
660-690	33	8	2	29.0	0.069	0.931	0.5673 (0.0524)	0.0024 (0.0017)
690-720	23	12	0	17.0	0.0	1.0	0.5282 (0.0556)	0.0 (0.0)
720-750	11	4	1	9.0	0.111	0.889	0.5282 (0.0556)	0.0039 (0.0039)
750-780	6	3	1	4.5	0.222	0.778	0.4695 (0.0742)	0.0083 (0.0083)
780-810	2	2	0	1.0	0.0	1.0	0.3652 (0.1086)	0.0 (0.0)

Table VI-2. Results of survival analysis of detection rates of bovine leukemia virus infection.

Variable	Values of variable	Chi Square (1 d.f.) Variable by age*	Variable alone
Dam parity	0,1	70 ($p < 0.0001$)	0.10 ($p = 0.75$)
Dam age (months)	20-120	79 ($p < 0.0001$)	0.07 ($p = 0.79$)
Dam status	0,1	35 ($p < 0.0001$)	0.54 ($p = 0.46$)
Colostrum	0,1	55 ($p < 0.0001$)	0.45 ($p = 0.50$)
Breed	0,1	85 ($p < 0.0001$)	0.03 ($p = 0.86$)

*Age at detection.

Table VI-3. Correlation coefficients of factors examined for association with infection with bovine leukemia virus (BLV).

Factor	Factor			
	Dam BLV status	Dam age	Colostrum*	Breed
Dam parity	0.30 (0.0001)+ n = 473	0.68 (0.0001) n = 470	0.18 (0.001) n = 332	-0.07 (0.11) n = 473
Dam BLV status		0.27 (0.0001) n = 471	0.81 (0.0001) n = 333	0.07 (0.15) n = 474
Dam age			0.18 (0.001) n = 333	-0.05 (0.29) n = 471
Colostrum*				0.14 (0.013) n = 333

*Refers to calves consuming colostrum with BLV antibodies.

+Figure in parentheses is the probablility >|R| under Ho: R = 0.

Table VI-4. Correlation coefficients of estimated parameters of hazard rate functions for the detection of bovine leukemia virus infection.

	Variable			
Variable	Dam status	Dam age	Colostrum	Breed
Dam parity	-0.002	-0.973	0.027	0.087
Dam status		-0.027	-0.857	-0.021
Dam age			-0.036	-0.052
Colostrum				-0.037

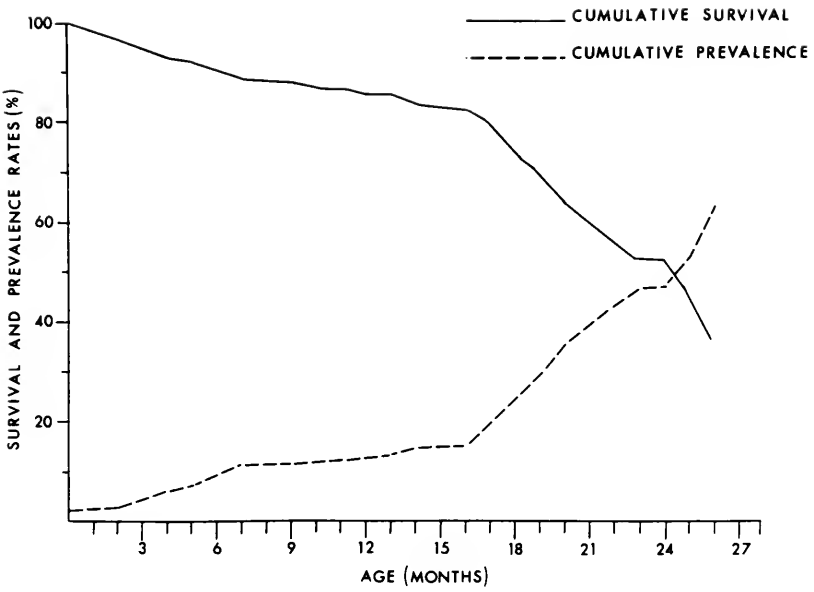


Fig. VI-1. Estimated age-specific cumulative survival (%) and prevalence rate (%) of detection of bovine leukemia virus infection.

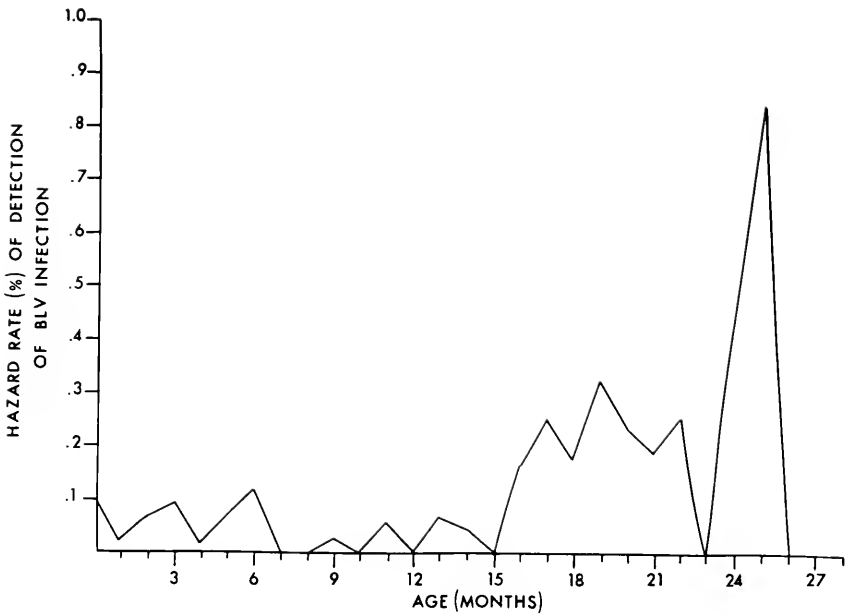


Fig. VI-2. Age-specific hazard rates of detection of bovine leukemia virus (BLV) infection.

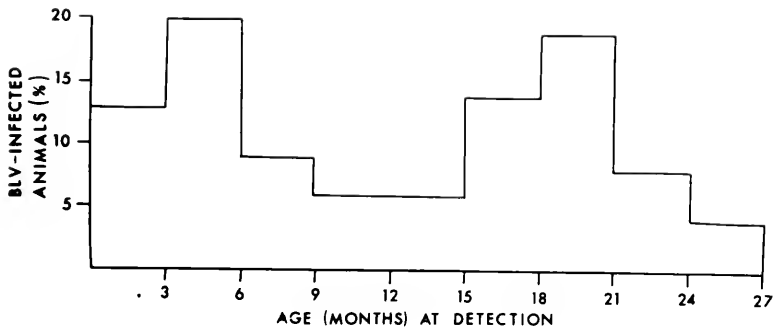


Fig. VI-3. Age at detection of bovine leukemia virus (BLV) infection for 63 BLV-infected animals.

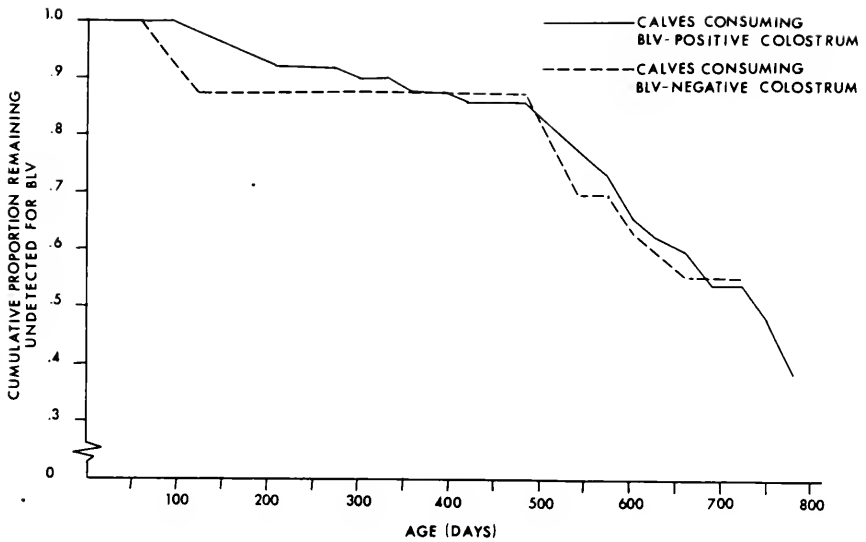


Fig. VI-4. Cumulative proportion of calves consuming colostrum with bovine leukemia virus (BLV) antibodies and of calves not consuming colostrum with BLV antibodies remaining undetected for BLV infection.

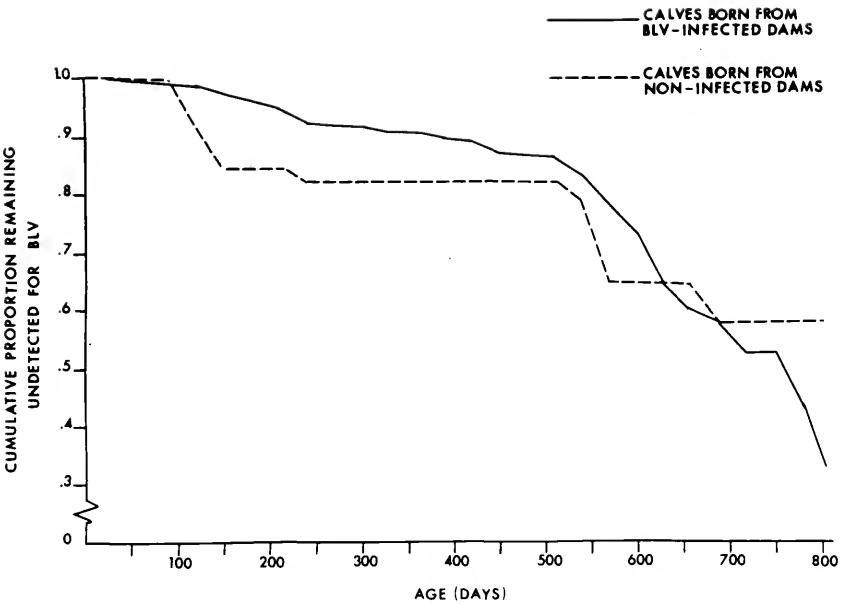


Fig. VI-5. Cumulative proportion of animals from bovine leukemia virus (BLV)-infected dams and from noninfected dams remaining undetected for BLV infection.

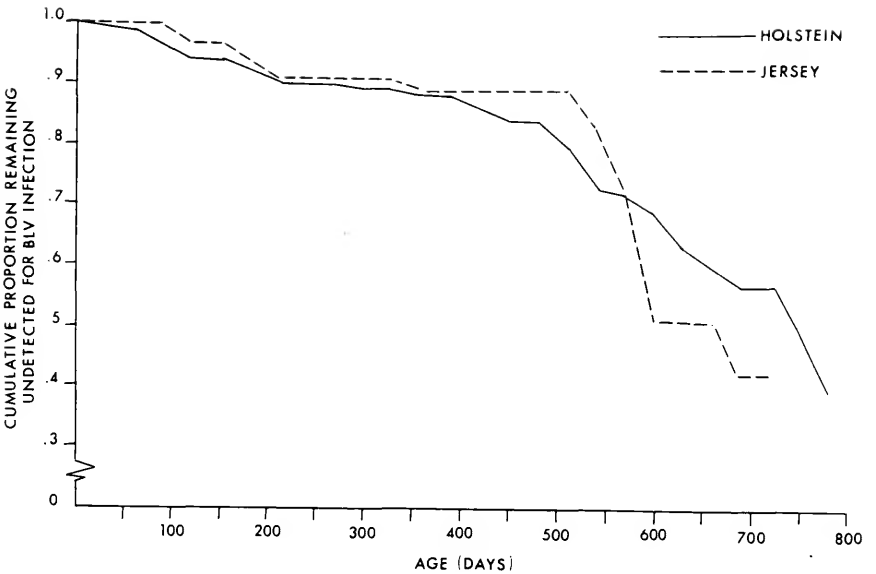


Fig. VI-6. Cumulative proportion of Holsteins and Jerseys remaining undetected for bovine leukemia virus (BLV) infection.

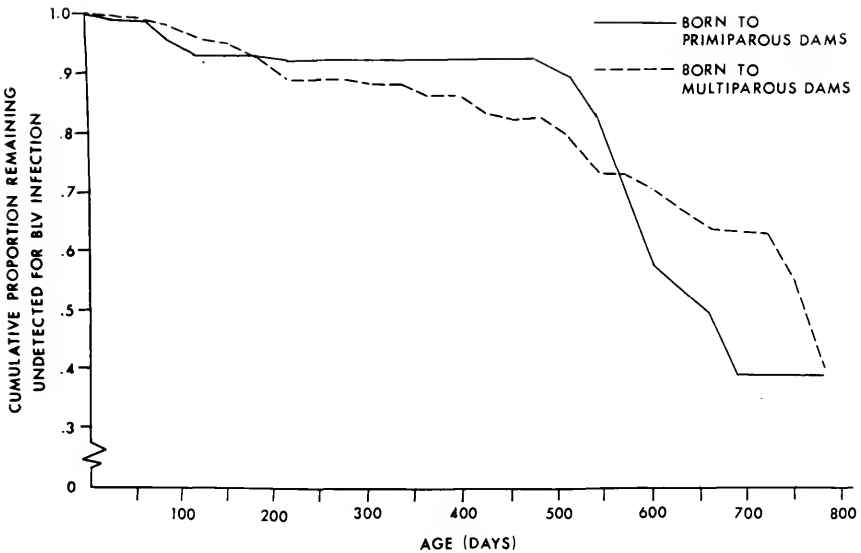


Fig. VI-7. Cumulative proportion of animals born to primiparous cows and of animals born to multiparous cows remaining undetected for bovine leukemia virus (BLV) infection.

CHAPTER VII
SEASONAL PATTERNS OF RATES OF BOVINE LEUKEMIA VIRUS INFECTION

Introduction

A fundamental consideration in the epidemiology of infectious diseases is the examination for temporal patterns of occurrence of a disease. Seasonal or secular trends in incidence rates may suggest modes of transmission, causal associations, and/or etiologic agents. If, for example, a hypothesized factor in disease transmission demonstrates seasonal variability, and a statistically significant seasonal cycle of disease exists in phase with the hypothesized factor, then supporting evidence favoring involvement of the factor in transmission has been demonstrated. On the other hand, if no seasonal occurrence of disease can be demonstrated, or it is not in phase with that of the factor, then the hypothesis of factor association should be rejected.

Interest in possible vector-borne transmission of BLV has arisen from evidence for arthropod involvement in the spread of another retrovirus, equine infectious anemia virus (Fischer et al., 1973; Hawkins et al., 1973a, 1973b). Reports based on experimental and observational data have suggested the involvement of arthropod vectors in BLV transmission (Bech-Nielsen et al., 1978; Ohshima et al., 1981).

Another study, however, revealed higher rates of infection during winter months, coinciding with crowded, indoor housing conditions rather than exposure to large numbers of flies (Wilesmith et al., 1980).

In this chapter, the hypotheses tested are as follows: (1) age-specific rates of detection of BLV infection do not differ with respect to month of birth; (2) rates of detection of BLV infection show no significant seasonal pattern associated with the seasonal frequency of potential arthropod vectors; and (3) no other seasonal pattern of rates of detection exists.

Materials and Methods

Month of Birth

Cox's hazard model described in CHAPTER VI was used to examine for an association between month of birth and subsequent BLV infection. Month of birth was denoted by z in the model. Detection of BLV infection was based on criteria presented in CHAPTER VI.

Month of Detection of BLV Infection

Monthly incidence rates of detection of BLV infection were computed to estimate seasonal patterns of BLV infection rates. Monthly incidence rates of detection were calculated as the number of animals detected during the month divided by the number of animals at risk of being detected

in that month. The criteria for an animal to be at risk for any month of follow-up were (1) the animal must not have been detected previously; (2) the animal must have been present for at least half of a month; (3) an animal born in the last half of a month was considered only in subsequent months; and (4) an animal remaining undetected for more than one year would be considered also for each repeated month. For example, if an animal were present during January 1980, and January 1981, it would be counted as two animals-at-risk during the month of January. Those animals detected as infected during a month also were counted in the at-risk denominator.

Possible differences between the 12 monthly incidence rates were examined using a Chi Square test with 11 degrees of freedom. In order to determine if monthly incidence rates followed a simple harmonic trend, a test for goodness-of-fit of the data to a simple harmonic curve was performed using a Chi Square statistic, as previously described (Walter and Elwood, 1975). The test for a simple harmonic trend of incidence rates, however, could not be pursued because a high Chi Square value ($X^2_{(11)} = 17.5$, $0.05<p<0.10$) indicated that these rates were inappropriately described by a simple harmonic curve.

An alternate, nonparametric approach to the seasonality of events was used to examine for six-month trends in incidence rates (Hewitt et al., 1971) Rates were computed only for animals over 12 months of age. The tested

hypothesis stated that six-month cumulative ranks of incidence rates from May through October, the fly season, were not greater than those for the 11 other six-month periods. A second hypothesis was tested for no differences in cumulative ranks of incidence rates for any nonspecified six-month period.

Results

Month of Birth

No significant differences were found between hazard rates of detection of BLV infection for animals born in each of the 12 months ($p = 0.24$). The proportion of animals infected was highest in animals born in August, September, and October (Fig. VII-1).

Month of Detection of BLV Infection

No significant differences were found between the 12 monthly incidence rates ($X^2_{(11)} = 9.81$, $0.4<p<0.5$).

The numbers of animals detected as BLV infected and at risk of detection for each of the 27 months of observation are presented in Table VII-1. Monthly incidence rates of detection for animals of all ages ranged from 1.1% in January and April to 3.0% in June (Fig. VII-2). For animals over 12 months of age, cumulative ranks of monthly incidence rates between May and October were not significantly greater than those for any other six-month period

($p = 0.20$) (Table VII-2). Cumulative ranks of monthly incidence rates were highest for the six-month period beginning with March and were lowest for the period beginning with September. The probability of cumulative ranks was lowest for the period beginning with March, but this was not significant without an a priori commitment ($p = 0.13$).

Discussion

Although no statistical significance could be placed on differences between birth-cohort hazard rates, calves born between August and October constituted 65% of the infected animals, even though only 42% of calves were born during that period (APPENDIX C). This was not surprising for several reasons. The density was highest during the period in which these calves were in the calf barn (CHAPTER III). In a crowded situation, transmission may occur either by aerosol (Van Der Maaten and Miller, 1978c) or by contact (Dechambre et al., 1968; Maas-Inderwiesen et al., 1978; Miller and Van Der Maaten, 1978a; Straub, 1971, 1978a; Wilesmith et al., 1980). Also, since these animals were the oldest in the study, they had a greater chance of entering the dry herd where there may have been an increased risk of infection due to contact with older, BLV-infected cattle. Calves born during the winter and spring were too young to be bred and enter the dry herd by May 1981. The proportion of calves born in the spring and

subsequently becoming infected would be expected to be low because few cows calved at that time (APPENDIX C).

Two assumptions were made in the analysis of monthly incidence rates of detection of BLV infection. One was that animals were considered independent within and among months followed. This is reasonable because exposure to a possible BLV-carrying insect and susceptibility to BLV infection were not likely to be related to any previous experience.

The other assumption in examination of monthly incidence rates was that any pattern of detection would be synchronized with that of infection and would consistently follow infection by two to three months. This assumption may not be valid for calves less than six months of age. Infection may occur in utero or shortly before birth but before acquisition of passive protection from colostral antibodies. In such cases, detection of infection may not be made for six months or more because virus repression may be dependent on both antibody and virus concentration (Driscoll et al., 1977; Straub, 1978a). It is believed, however, that viral expression would appear within 12 months of infection (Van Der Maaten et al., 1981a).

Analysis of possible vector transmission also may have been confounded by calfhood management. Since the calving season occurred during the summer and early fall, animal density in the calf barn was highest during late summer and fall. Seroconversion of animals several months later could

be interpreted as infection resulting from close physical contact rather than from arthropod vectors. Furthermore, the risk of infection for calves in the calf barn may not have been as high as for those over one year of age. Flies found in the calf barn were not usually the biting type, and sporadic insect control was practiced, which limited exposure to potential vectors. Colostral antibodies also may have provided some resistance to infection from small doses of virus introduced by an insect (Van Der Maaten et al., 1981a).

The situation with cattle more than 12 months of age was quite different. Biting flies were present on these animals throughout summer months, and no fly control was practiced. Prevalence rates of infection also were higher in these animals (CHAPTER VI), increasing the chance that a fly would first feed on an infected animal. Cattle over one year of age, therefore, were analyzed separately as they constituted a more susceptible and exposed population to possible vector-borne transmission and because infection was more easily detected and analysis was not confounded by management factors.

Results of the Chi Square test indicated that there were no significant differences between monthly incidence rates. Important seasonal trends, however, may be present without apparent rate differences between months (Walter and Elwood, 1975). The method proposed for analysis of seasonal trends in incidence rates (Edwards, 1961; Walter

and Elwood, 1975) was particularly appealing because seasonal frequencies of potential arthropod vectors in temperate and subtropical climates generally resemble simple harmonic curves which peak during summer months (Beck, 1958; Blanton and Wirth, 1979; Bohart and Washino, 1978; Jones and Anthony, 1964; Khalaf, 1969). Their analysis could not be pursued because the incidence rates were inappropriately described by a simple harmonic curve. The goodness-of-fit test indicated that incidence rates did not resemble a simple harmonic curve and no further tests for significance or maximum amplitude of a curve should be performed. This analytic method also could not be used to test rates in older animals because the recommended sample size is not less than 50 (Walter and Elwood, 1975). Instead, a nonparametric method appropriate for small samples was used to examine for excessive rates over six-month periods (Hewitt et al., 1971).

Difficulties with Hewitt's alternative procedure have been its lack of power and inability to estimate parameters of an harmonic curve (Walter and Elwood, 1975). In spite of these restraints and the few infections detected, a marked tendency for maximum risk of detection of infection was revealed between March and August ($p = 0.13$), the six-month period beginning with March. Had an a priori hypothesis been specified for that particular yearly segment, there would be reason to believe that an excessive rate of detection had occurred during that period ($p = 0.01$). This interval would coincide with infection resulting from

exposure to animals in the dry herd between January and May 1981. If vectors had played a major role in transmission and assuming peak infection around July, then maximum cumulative ranks of incidence rates of detection should have been observed between May and October. This was not the case, as indicated by acceptance of the hypothesis of no increase in incidence rates for that six-month period.

Results of this study are consistent with those from a study in which increased rates of seroconversion were observed after cattle grazed together on summer pastures (Onuma et al., 1980). In that study, arthropod-borne transmission could not be examined because analysis would be confounded by congregation of cattle only during the vector season. In another study, two groups were compared for rates of infection during winter and summer months (Bech-Nielsen et al., 1978). Rates observed for summer and winter groups, 4/7 and 1/7, respectively, could have occurred by chance (Fisher's exact $p = 0.13$). A study of incidence rates of BLV seroconversion in a large dairy found higher rates in winter and spring, implying transmission associated with crowded housing (Wilesmith et al., 1980). None of these studies examined monthly rates for a complete seasonal cycle. Future studies of seasonal patterns of disease should be based on at least two years of monthly cohort data in an attempt to remove as much year-effect as possible.

Experimental studies have attempted to examine potential horsefly transmission of BLV. After finding BLV in the midgut of Tabanus nigrovitatus, it was concluded that fly control may reduce transmission rates (Bech-Nielsen et al., 1978). However, horseflies are interrupted and inefficient feeders and only transmit disease agents mechanically via their mouthparts (James and Harwood, 1979). Presence of BLV in the midgut of a tabanid probably provides little evidence that these flies are natural vectors of BLV. Another study which used various species of horseflies demonstrated transmission of BLV to sheep (Ohshima et al., 1981). Flies which had fed on an infected cow were manually transferred to noninfected sheep and held on the sheep for as many as 140 feedings in four days. Although proving that horseflies can transmit BLV to an unnatural host, the question remains concerning the extent to which these flies transmit BLV infection to cattle under natural conditions.

Circumstantial evidence favors the hypothesis that vectors do not contribute significantly to BLV transmission. Beef herds have much lower BLV-infection rates than dairy herds (Baumgartener et al., 1975; Burridge et al., 1981; House et al., 1977). Fly control usually is more intense in dairy than beef herds partially because of milk sanitation requirements. If biting flies were important in transmission, these differences between rates probably would not be observed.

Incidence rates of detection of BLV infection observed in cattle over 12 months of age suggested no association with frequencies of potential arthropod vectors. Trends in incidence rates were suggestive of a management procedure, however, specifically that of moving heifers into a population of older, infected cattle. Therefore, special attention should be paid to management interventions whenever examining seasonal rates of animal infections.

Table VII-1. Numbers of animals detected as infected with bovine leukemia virus and numbers at risk of detection for each month of observation.

Year	Month	Number at risk	Number detected	Incidence rate (%)	Year	Month	Number at risk	Number detected	Incidence rate (%)
1979	July	2	0	0	1980	Aug	127	1	0.8
	Aug	8	0	0		Sept	150	0	0
	Sept	39	0	0		Oct	166	3	1.8
	Oct	64	0	0		Nov	178	4	2.2
	Nov	85	3	3.5		Dec	186	1	0.5
	Dec	71	2	2.8	1981	Jan	199	1	0.5
1980	Jan	80	2	2.5		Feb	205	3	1.5
	Feb	79	1	1.3		Mar	199	4	2.0
	Mar	85	3	3.5		Apr	182	3	1.6
	Apr	84	0	0		May	179	4	2.2
	May	86	1	1.2		June	177	8	4.5
	June	88	0	0		July	158	4	2.5
	July	108	0	0		Aug	145	4	2.8
						Sept	135	2	1.5

Table VII-2. Monthly incidence rates of detection of bovine leukemia virus infection in animals between 12 and 27 months of age.

Month	Number detected (1)	Number at risk (2)	Incidence rate (%) (1) ÷ (2)	Rank of incidence rate	Cumulative ranks for next six months	Probability*	
						Any six-month segment	Specified six-month segment
Jan	0	55	0	1.5	45.5	0.87	0.18
Feb	2	63	3.17	7	49.0	0.50	0.07
Mar	3	67	4.47	9	53.0	0.13	0.01
Apr	3	66	4.54	10	47.0	0.73	0.12
May	2	65	3.08	6	45.0	0.91	0.20
June	6	62	9.68	12	43.0	0.99	0.29
July	2	72	2.78	5	32.5	1.00	1.00
Aug	4	81	4.94	11	29.0	1.00	1.00
Sept	1	91	1.10	3	25.0	1.00	1.00
Oct	1	31	3.23	8	31.0	1.00	1.00
Nov	1	41	2.44	4	33.0	1.00	1.00
Dec	0	50	0	1.5	35.0	1.00	1.00

*From Hewitt et al., 1971.

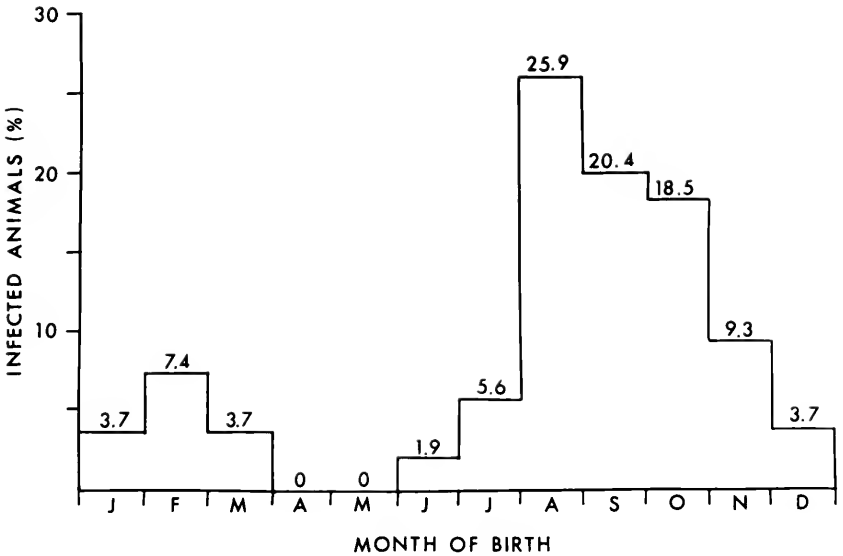


Fig. VII-1. Percentage of 54 animals infected with bovine leukemia virus born in each of the 12 months of the year.

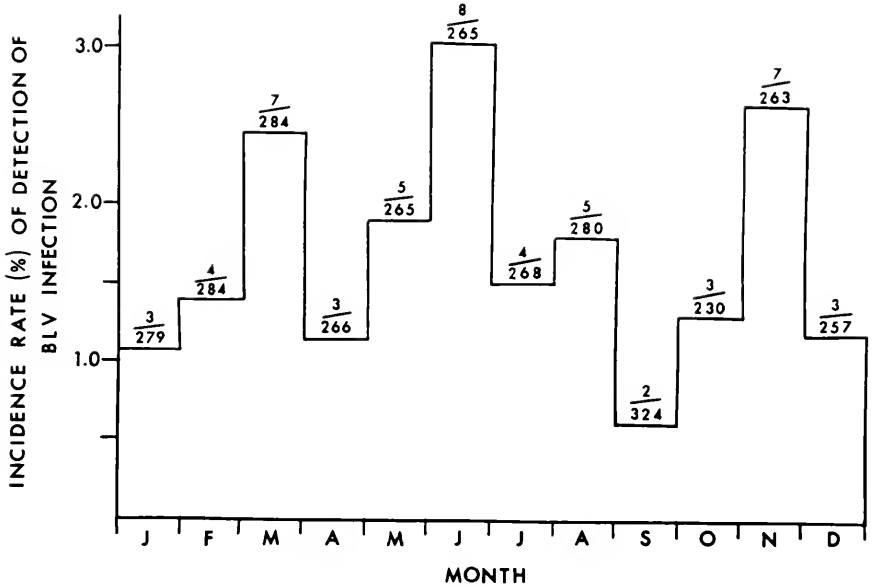


Fig. VII-2. Monthly incidence rates (%) of detection of bovine leukemia virus (BLV) infection in 54 animals. Figures above bars are the number of animals detected divided by the number of animals at risk.

CHAPTER VIII
SPATIAL PATTERNS OF BOVINE LEUKEMIA VIRUS INFECTION

Introduction

In previous chapters, patterns of BLV infection were studied by examining age or temporal trends in detection of BLV infection. Assuming servoconversion actually occurred within about two months of infection, infection patterns could be examined by a two-month temporal adjustment in detection rates. Age-specific rates and seasonal patterns of detection both suggested a strong association between infection and exposure to the dry herd. A common location at infection, however, may become obscured as animals move through various pastures, thus making it difficult to examine for a hypothesized spatial component of transmission. An animal, therefore, may have been detected as infected in the dry herd when it was actually infected in some prior location.

Another aspect of spatial associations concerns transmission of BLV infection in the calf barn. Calves may have occupied a pen adjacent to an infected calf, possibly increasing the risk of infection through imposed close contact.

The intent of this chapter is two-fold. Firstly, a hypothesis is tested which states that, while in the calf

barn, rates of detection do not differ between calves penned adjacent to calves infected in utero and calves not in adjacent pens. Secondly, to assess risk of BLV infection at various locations, weighted expected probabilities of BLV infection are derived using a seroconversion function employing results from experimental studies.

Materials and Methods

Risk of BLV Infection from Exposure to Calves Infected In Utero

Calves known to be infected while in the calf barn were the eight calves previously identified as infected in utero (CHAPTER IV). Calf H949 also was included in this group because of evidence of in utero or very early postnatal infection (Table V-1). Exposed animals were defined as calves which had been located adjacent to any of the nine calves mentioned above while in the calf barn. Evidence for BLV infection in exposed and nonexposed calves was based on criteria established in CHAPTER VI. A hypothesis of no association between exposure to the nine infected calves and subsequent infection was tested using Fisher's exact test from STATPAK of MUSIC. Associations were examined for the average number of calves followed for six months and for the average number followed for twelve months. Averages were computed by dividing by two the sum of the number of calves beginning the follow-up period and the number present at the end of the period.

Risks of BLV Infection in Five Types of Locations

Weighted expected probabilities of infection

A. Seroconversion function. From previously published data (Mammerickx et al., 1980; Van Der Maaten and Miller, 1978a, 1978b), a seroconversion function was derived which described the probability of an animal having BLV antibodies for each day following the experimental inoculation of BLV. Cumulative proportions of animals serologically positive were calculated for each day after inoculation. By dividing the weekly proportions (differences between consecutive weekly cumulative rates) by seven, an expected daily proportion was obtained for that week (Table VIII-1). Daily probabilities, $\{p_i\}_{\ell=1}^{52}$, were then derived from an approximated curve plotted through these week midpoints (Fig. VIII-1), where the probability of seroconversion by 52 days postinoculation was 0.9996 (Table VIII-2). Cumulative probabilities, $\{P_i = \sum_{\ell=1}^{i} p_\ell\}_{i=1}^{52}$, were obtained by summation of the p_ℓ.

B. Algorithm for expected probabilities associated with locations. Utilizing the cumulative function, P_i, an algorithm was devised which examined location-specific infection rates. The algorithm assigned fractions of infection to locations occupied by the infected animal prior to detection of infection. These fractions were totaled for each of five locations and divided by the animal-days of occupancy to arrive at an expected rate of infection

for each location. These rates were then compared to those based only on location at the time of detection of infection.

Expected probabilities for each location were derived by first computing the probability of infection for each of the 52+k days prior to detection of an animal, given that a negative test was observed k days prior to the positive test (detection).

Two vectors, A and B, were defined such that

$$\underline{A} = \{A_i\} = \{P_1, P_2, \ldots, P_{52}, \underbrace{1, 1, \ldots, 1}_{k}\}_{52+k},$$

where P_i was the cumulative probability described above and 1 was the probability of infection for each of the k days between the negative test and detection, and

$$\underline{B} = \{B_i\} = \{\underbrace{1, 1, \ldots, 1}_{k}, 1-P_1, 1-P_2, \ldots, 1-P_{52}\}_{52+k},$$

where $1-P_i$ was the cumulative probability of being infected within i days prior to detection, and 1 was the probability of being infected for each of the k days prior to the negative test. Conditional probabilities were estimated by

$$\underline{AB} = \{A_i B_i\}_{i=1}^{52+k}$$

and in turn were weighed by

$$1/\sum_{i=1}^{52} A_i B_i = 1/\underline{A}\,\underline{B}^t$$

to obtain the proper density function, where B^t is the transpose of the B vector. These functions would appear as

$$F(52+k) = \{A_i B_i\}/\underline{A}\ \underline{B}^t = 0 ,$$

$$F(52+k-1) = \sum_{i=52+k-1}^{52+k} \{A_i B_i\}/A\ B^t ,$$

.

.

$$F(1) = \sum_{i=1}^{1} \{A_i B_i\}/\underline{A}\ \underline{B}^t ,$$

$$F(0) = 1.$$

Once density functions were defined for each animal, fractions of infection, P_j', were allocated to each of j locations according to time of occupancy in the 52+k days prior to detection. Time was measured from detection such that t_j was the number of days from entering location j until detection, where the location at detection was j = 1 (Fig. VIII-2). Probabilities of an animal becoming infected in each of j locations, for example, would be

$$P_1' = F(0) - F(t_1),$$

$$P_2' = F(t_1) - F(t_2),$$

.

.

$$P'_j = F(t_{j-1}) - F(t_j) .$$

Risks associated with locations

Locations, grouped by type of management, were calf barn (indoor, outdoor, and combined), paddocks, calf unit, heifer pastures, and dry herd. An estimated risk of infection was calculated for a location by dividing the sum of the P''s for that location by the animal-days of occupancy in that location. Animal-days was calculated by summing days spent in the location for each animal remaining undetected for BLV infection. Rates of detection for each location were calculated as the number of animals detected in the location divided by the animal-days of occupancy.

Relative and attributable risks of expected infection rates in locations were computed and probabilities of relative risks equaling one were calculated as previously described (Schwabe et al., 1977).

Location prevalence rates

A typical prevalence rate of BLV infection for the dry herd was estimated from all cattle occupying the dry herd between early January 1981 and September 30, 1981. The rate was calculated as the number of BLV-seropositive cattle divided by the total number of animals occupying the dry herd during that period. Prevalence rates for other locations were estimated from the high range of age-specific

rates (Fig. VI-1) for the age group in each location (CHAPTER III).

Results

Risk of BLV Infection from Exposure to Calves Infected In Utero

No significant association was found between exposure in the calf barn to calves infected with BLV in utero and subsequent BLV infection detected either prior to six months of age ($p = 0.61$) or prior to one year of age ($p = 0.63$) (Table VIII-3).

Risk of BLV Infection in Five Types of Locations

The sum of weighted, expected probabilities of BLV infection for each location for 53 infected animals is presented in Table VIII-4. The estimated number of animals infected per 10,000 animal-days at risk ranged from 2.26 in the calf unit to 29.39 in the dry herd (Table VIII-4). Significantly high relative risks were associated with the dry herd compared to the calf barn ($0.0001<p<0.001$), paddocks ($0.001<p<0.05$), calf unit ($p<0.001$), and heifer pastures ($p<0.001$) (Table VIII-5). A significantly low relative risk was associated with the calf unit compared to the calf barn ($p<0.05$).

The number of infections per 10,000 animal-days at risk attributed to the dry herd location ranged from 27.1

compared to the calf unit to 23.2 compared to the calf barn (Table VIII-5).

Rates of detection of BLV infection in each of the five location types is presented in Table VIII-6.

Location Prevalence Rates

A typical dry herd prevalence rate of BLV infection was estimated to be 76%. Rates for the calf barn, paddocks, calf unit, and heifer pastures were 5%, 12%, 12%, and 20%, respectively.

Discussion

No increased risk of infection was observed in calves penned next to calves infected *in utero*. Colostral antibodies may have repressed viral replication and subsequent virus shedding due to immunologic modulation, as discussed previously (CHAPTER VI). Some resistance to infection also may have been offered to the noninfected calf by colostral antibodies. As colostral antibodies decay, however, transmission of infection may be successful. The early detection and isolation of infected calves, therefore, should be encouraged.

Estimated relative and attributable risks presented a clear indictment for exposure to the dry herd as a major factor influencing BLV transmission. Further support was provided for the hypothesis that physical contact is necessary before transmission can take place (Maas-Inderwiesen

et al., 1978; Miller and Van Der Maaten, 1978a; Ferrer and Piper, 1981; Wilesmith et al., 1980), because heifers in pastures differed from those in the dry herd only by their exposure to a lower prevalence rate of infection (20% versus 76%).

Increased risk in the dry herd may have resulted not only from exposure to a high percentage of infected cattle but also from increased contact due to high animal density, as suspected in another study (Wilesmith et al., 1980). Similarly, the drop in risk associated with the calf unit compared to the calf barn or paddocks may have represented a reduction in animal density. Further examination of interactions of prevalence rate and density on transmission rates of BLV infection deserves attention, particularly in establishing guidelines for BLV control.

Some quantitative guidelines for the use of five location types in heifer management were provided here by risk estimates. It is shown, for instance, that heifers would experience a risk of BLV infection in the dry herd over five times that in heifer pastures. Over a three month period (about 100 days), this would amount to 24 of 100 heifers infected due to exposure in the dry herd. Only a slight protection from infection was associated with use of outdoor calf pens. Because few calves occupied the outdoor pens, numbers may be too small for practical comparisons.

These risk estimates could help set cost-effective priorities in managing BLV infection in a dairy. They also

could be used to identify other sources of transmission by focusing on reasons why locations are associated with different risks of infection.

The algorithm presented here retrospectively partitions the likelihood of an animal becoming infected with BLV, starting from the time of detection. The estimated fractions of infection for any given time interval have two components. One is the constant seroconversion function, derived here from results of three experimental studies. The other component is the time between tests, a variable.

Improvement in the validity of the algorithm, and thus proportions estimated, could be made by improving either of these components. The seroconversion function could be refined by using rates of seroconversion from a large sample of animals experimentally infected by a method simulating natural infection (e.g., perhaps aerosol) and tested at daily intervals. Further improvements in estimated proportions could be made by shortening the testing interval (k) of animals under study, thus reducing the estimated period of transmission. Methods for measuring bounds on the error of these estimates also would be useful.

This technique could benefit studies of other diseases in which infection precedes signs or symptoms by several weeks or months. A measure of confidence would be provided for infection in some time period prior to detection or diagnosis of the disease.

More appropriate tests of hypotheses could be made for factors observed during a period preceding detection rather than for factors observed at the time of detection. This was illustrated by the discrepancy between risks measured at the time of detection of BLV infection and those estimated prior to detection. The latter method provided more insight into infection occurring at young ages, as demonstrated by higher risks associated with the calf barn and paddocks.

Table VIII-1. Summary of three studies on experimental infection with bovine leukemia virus (BLV) from which probabilities for seroconversion were derived as a function of days postinoculation with BLV.

		Days after inoculation with BLV							
	Study*	0	7	14	21	28	35	42	49
Number of animals present	a	9	9	9	9	9	9	9	9
	b	5	5	4	3	3	2	2	2
	c	6	6	6	6	6	6	6	6
Total (1)		20	20	19	18	18	17	17	17
Number of animals serologically positive for BLV	a	0	0	1	2	4	6	8	9
	b	0	0	0	0	0	1	2	2
	c	0	0	0	1	3	4	6	6
Total (2)		0	0	1	3	7	11	16	17
Cumulative proportion (3) = (1) ÷ (2)		0	0	0.053	0.167	0.389	0.647	0.941	1.0
Weekly proportion (4)			0	0.053	0.114	0.222	0.258	0.294	0.059
Daily proportion at midweek (5) = (4) ÷ 7			0	0.0076	0.0163	0.0317	0.0369	0.0420	0.0084

*a. Van Der Maaten and Miller, 1978a.

b. Van Der Maaten and Miller, 1978b.

c. Mammerickx et al., 1980.

Table VIII-2. Daily and cumulative daily probabilities of seroconversion to bovine leukemia virus (BLV) following experimental inoculation with BLV.

Days postinoculation	Probability of seroconversion*	
	Daily (p_i)	Cumulative daily (P_i)
1	0.0	0.0
2	0.0	0.0
3	0.0	0.0
4	0.0003	0.0003
5	0.0011	0.0014
6	0.0018	0.0032
7	0.0028	0.0060
8	0.0039	0.0099
9	0.0051	0.0150
10	0.0063	0.0213
11	0.0073	0.0286
12	0.0088	0.0374
13	0.0101	0.0475
14	0.0114	0.0589
15	0.0130	0.0719
16	0.0146	0.0865
17	0.0162	0.1027
18	0.0179	0.1206
19	0.0197	0.1403
20	0.0216	0.1619
21	0.0233	0.1852
22	0.0251	0.2103
23	0.0267	0.2370
24	0.0286	0.2656
25	0.0302	0.2958
26	0.0318	0.3276
27	0.0332	0.3608
28	0.0348	0.3956
29	0.0358	0.4314
30	0.0367	0.4618
31	0.0377	0.5058
32	0.0385	0.5443
33	0.0392	0.5835
34	0.0400	0.6235
35	0.0404	0.6639
36	0.0408	0.7047
37	0.0411	0.7458
38	0.0416	0.7874
39	0.0418	0.8292
40	0.0393	0.8685
41	0.0328	0.9013
42	0.0257	0.9270

Table VIII-2. Continued.

Days postinoculation	Probability of seroconversion*	
	Daily (p_i)	Cumulative daily (P_i)
43	0.0197	0.9467
44	0.0148	0.9615
45	0.0112	0.9727
46	0.0078	0.9805
47	0.0065	0.9870
48	0.0047	0.9917
49	0.0037	0.9954
50	0.0023	0.9977
51	0.0014	0.9991
52	0.0005	0.9996

*Determined by AGID-gp in experimental studies (Mammerickx et al., 1980; Van Der Maaten and Miller, 1978a, 1978b).

Table VIII-3. Association of bovine leukemia virus infection (BLV) with exposure in the calf barn to calves infected with BLV in utero.

Exposure of calves	Calves followed for 6 months			Calves followed for 12 months		
	Number of calves			Number of calves		
	Infected	Not infected	Total*	Infected	Not infected	Total*
In pen adjacent to a calf infected in utero	2+	18	20	2±	14	16
In pen not adjacent to a calf infected in utero	22	203	225	26	161	187

*Average number of calves followed in the follow-up period.

+Exact p = 0.61.

±Exact p = 0.63.

Table VIII-4. Estimated risk of infection with bovine leukemia virus (BLV) associated with locations occupied by animals prior to detection of BLV infection.

Location type	Expected number of animals infected ($\Sigma P'_j$) (1)	Animal-days of occupancy (2)	Estimated risk of infection (animals per 10,000 animal-days at risk) (1) ÷ (2)
Calf barn			
indoor	10.57	16,729	6.32
outdoor	2.70	4,727	5.72
combined	13.28	21,456	6.19
Paddocks	3.09	5,280	5.85
Calf unit	6.46	28,658	2.26
Heifer pastures	20.25	38,518	5.26
Dry herd	9.92	3,376	29.38
	53.00		

Table VIII-5. Relative and attributable risks of infection with bovine leukemia virus (BLV) estimated for five location types.

	Location type				
	Calf barn				
Location type	Outdoor	Paddocks	Calf unit	Heifer pastures	Dry herd
Calf barn					
Indoor	0.91 (-0.6)*				
Combined		0.95 (-0.3)	0.37+ (-3.9)	0.85 (-0.9)	4.75± (23.2)
Paddocks			0.39 (-2.3)	0.90 (-0.6)	5.02† (23.5)
Calf unit				2.33 (3.00)	13.00‡ (27.1)
Heifer pastures					5.59‡ (24.1)

*Figure in parentheses is estimated attributable risk (number of BLV-infected animals per 10,000 animal-days at risk).

+Probability that relative risk = 1.0, $p<0.05$.

±$0.0001<p<0.001$.

†$0.01<p<0.05$.

‡$p<0.0001$.

Table VIII-6. Risk of detection of bovine leukemia virus (BLV) infection associated with locations occupied by animals at the time of detection.

Location type	Number detected (1)	Animal-days of occupancy (2)	Risk of detection (number detected per 10,000 animal-days at risk) (1) ÷ (2)
Calf barn			
Indoor	4	16,729	2.39
Outdoor	2	4,727	4.23
Combined	6	21,456	2.80
Paddocks	4	5,280	7.58
Calf unit	13	28,658	4.54
Heifer pastures	21	38,518	5.45
Dry herd	9	3,376	26.66
	53		

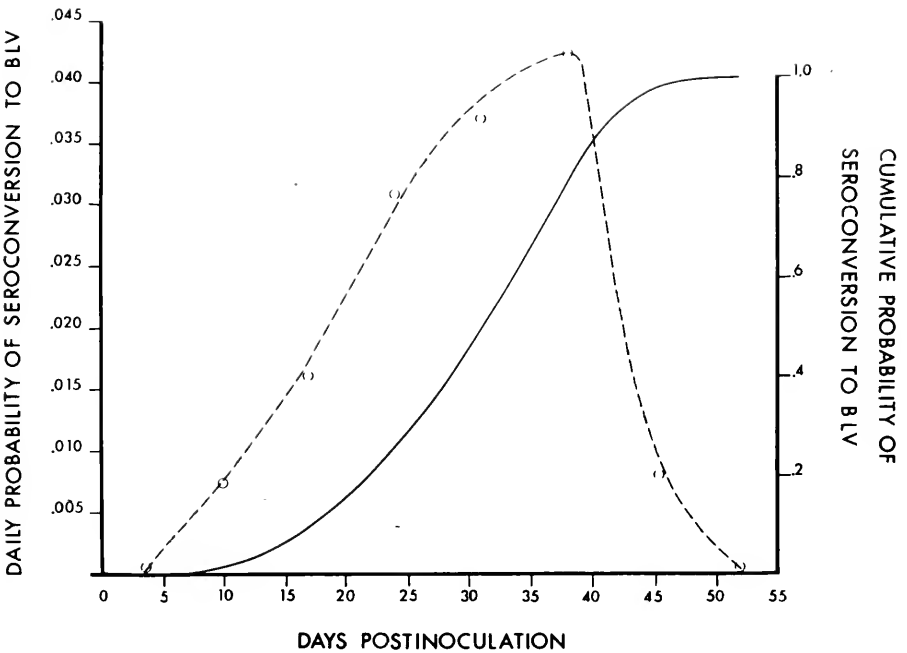


Fig. VIII-1. Daily (----) and cumulative daily (———) probabilities for appearance of antibodies to bovine leukemia virus (BLV) as a function of days postinoculation with BLV. Functions were derived from data presented by Mammerickx et al., 1980, Van Der Maaten and Miller, 1978a, 1978b.

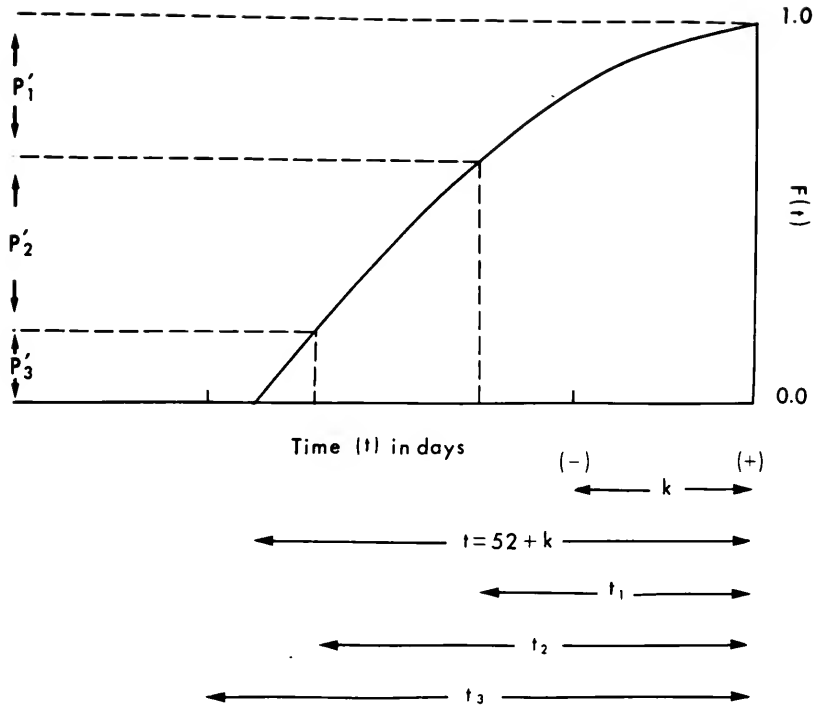


Fig. VIII-2. Hypothetical proper density function showing probabilities (P'_j) of an animal becoming infected with bovine leukemia virus (BLV) in each of three locations, j = 1, 2, and 3, prior to detection (+) of BLV infection which followed a negative test result (-) by k days.

CHAPTER IX
IATROGENIC TRANSMISSION OF BOVINE LEUKEMIA VIRUS INFECTION

Introduction

It was previously suggested that vaccination procedures contributed little, if anything, to transmission of BLV infection in the animals studied (CHAPTER VI). This conclusion was based on the low hazard rates of detection of BLV infection observed at ages in which vaccinations were administered. Because information on vaccinations was available, further investigation into iatrogenic transmission of BLV infection following routine vaccinations was warranted.

The purpose of this chapter is to test a hypothesis that the rate of detection of BLV infection was unchanged by vaccinations for other infectious diseases.

Materials and Methods

Infected calves were allocated to one of two groups, depending on the date of detection of BLV infection relative to dates of vaccinations. Calves detected within 90 days before a vaccination comprised one group, and those detected within 90 days after a vaccination the other group. An animal for which the 90-day intervals overlapped

was placed in the postvaccination group. Rates of detection of BLV infection for each group were calculated as the number of animals detected divided by the number of animal-vaccinations. Thus, an animal was considered for each vaccination as long as it was not detected as BLV infected within 90 days before or after a previous vaccination.

A z statistic for the ratio of the rate-after to the rate-before vaccination (the relative risk associated with vaccination) was calculated as

$$z = \frac{\log_e RR}{SE(\log_e RR)},$$

where $\log_e RR$ was the natural log of the relative risk, $\frac{a/a+b}{c/c+d}$, where a was the number detected and b the number remaining undetected after vaccination, and c the number detected and d the number remaining undetected prior to vaccination, and $SE(\log_e RR) = \sqrt{1/a+1/c-1/b-1/d}$ (Schwabe et al., 1977). The hypothesis tested was Ho: $\log_e RR = 0$, Ha: $\log_e RR > 0$.

Because assumptions of independence were violated by counting an animal more than once, a second analysis was performed which compared two independent groups. Rates of detection of BLV infection were examined in calves 90 days after brucellosis vaccination and in heifers 90 days before vaccination for viral diseases. Heifers in the latter group were the only ones vaccinated for infectious bovine rhinotracheitis, bovine virus diarrhea, and parainfluenza-3.

A hypothesis that detection rates after vaccination were no greater than before was tested using Fisher's exact test from STATPAK of MUSIC. This test calculated the probability of encountering frequencies as rare or rarer than those observed.

Results

A total of 270 animal-vaccinations were conducted during the 27-month study. Most of these (186) were for brucellosis (Table IX-1). Of the total vaccinated, 9 were detected within 90 days before a vaccination and 12 were detected within 90 days after a vaccination. One heifer, detected as infected in both a pre- and post-vaccinal period, was assigned to the postvaccination group. The relative risk of detection of BLV infection following vaccination was 1.38 (Table IX-2). The calculated z statistic was 0.74 ($p = 0.46$). For rates of detection of infection in the independent groups, Fisher's exact probability was 0.33 (Table IX-3).

Discussion

The design used in examination of relative risk may seem unorthodox because assumptions of independence were violated. A defense of this analysis of repeated measures rests in the supposition that an animal is equally susceptible to BLV infection before and after vaccination. Nevertheless, a second analysis was performed to examine

rates in independent groups. Fisher's exact test was used because at least one expected cell frequency was less than five.

The allocation of an infected heifer into a postvaccinal group interjected a bias against the null hypotheses. In spite of this maneuver, null hypotheses were accepted.

A 90-day interval was chosen for retrospective and prospective follow-up because studies have shown that seroconversion occurs within three months of natural infection (Straub, 1978b) and within seven weeks of experimental infection (Mammerickx et al., 1980; Van Der Maaten and Miller, 1978a, 1978b).

Factors potentially confounding a vaccination effect were present before brucellosis vaccination and after viral vaccinations. In utero and neonatal infection may surface after decay of colostral antibodies (Van Der Maaten et al., 1981b). This could result in more prevaccinal detection of infection in the brucellosis group since, by 131 days of age, colostral antibodies were no longer detectable in 90% of calves (CHAPTER V). Furthermore, risks of infection, such as exposure to infected animals in paddocks or calf pens, were not consistent before and after brucellosis vaccination. Similarly, following viral vaccinations heifers were inseminated and moved to the dry herd where risk of infection increased (CHAPTER VIII). These interventions would confound any postvaccinal effect. For these reasons independent groups were examined only after

brucellosis and before virus vaccinations. Management of heifers between these vaccinations did not appear to invoke any confounding effect.

These results, although based on small numbers, substantiate the interpretation of hazard rates of detection and infection proportions discussed previously (CHAPTER VI), as well as results of one experimental study (Roberts et al., 1981). In that study, transmission of BLV infection could not be demonstrated after alternately tuberculin-testing BLV-infected cows and susceptible calves or sheep. Infection did occur, however, when a drop of blood from a BLV-infected animal was placed on the needle prior to inoculation. Transmission may not have taken place in the present study because needles used were either too small to carry an infective dose of lymphocytes and/or withdrawal of the needle may have wiped off infective cells.

Other plausible explanations relate to prevalence rates of infection and passive immunity at the time of vaccination. The chance of inoculating a susceptible animal immediately after an infected animal would be less when prevalence rates were low. In addition, heifers vaccinated for brucellosis may have been partially protected from BLV infection because 10% still had detectable antibodies at 131 days of age. In one experimental study, however, colostral antibodies failed to protect against parenteral inoculation of BLV (Van Der Maaten et al., 1981a).

Another explanation for the decline in hazard rates of detection of infection observed during vaccination ages could be that vaccination or use of anthelmentics instilled a protective effect against BLV infection. Interferon has been stimulated by Brucella abortus extracts (Keleti et al., 1974; Kern et al., 1976) and by vaccination with infectious bovine rhinotracheitis virus (Cummins and Rosenquist, 1980). It has been shown that interferon suppressed viral replication of murine leukemia virus (Pitha et al., 1976) but had no effect on visna virus (Carroll et al., 1978). In addition, immunopotentiation has been observed in some studies following use of the anthelmentic levamisole (Irwin, 1976; Irwin et al., 1980); however, this has not been a consistent finding (Irwin et al., 1976).

Lymphocytotic state of donors also could play a role in needle transmission. The phenomenon of persistent lymphocytosis would enhance transmission via blood because increased numbers of BLV-infected lymphocytes become available in peripheral blood (Kenyon, 1976; Kenyon and Piper, 1977; Kettmann et al., 1980a; Kumar et al., 1978; Paul et al., 1977). Persistent lymphocytosis may be age related, with young animals less prone to develop the condition (Hofirek et al., 1978; Levy et al., 1977; Mammerickx et al., 1976b, 1977). This suggests that blood-borne transmission is less likely from young donors and may explain the low rates of detection of infection following vaccination of young cattle in this study.

It has been stated that any process which transmits blood from one animal to another probably provides a means of transmission of BLV infection (Van Der Maaten and Miller, 1978c). Vaccination for other infectious diseases provides a possible route of transmission through use of modified-live virus vaccines. The viral vaccines used during the course of this study were similar to those used in most dairy and beef herd health programs and are modified-live viruses derived from bovine cell lines. Contamination of cell lines with BLV has not been reported but should be considered until evidence proves otherwise.

Beef cattle are vaccinated routinely en masse for various diseases, usually on an annual basis. Programs and practices of vaccination in beef herds generally resemble those in dairy herds. If vaccination constituted a means of transmission, beef and dairy herds should have similar rates of infection, as discussed previously (CHAPTER VII).

Reports of iatrogenic transmission have referred only to procedures in which blood was sampled (Bause et al., 1978; Maas-Inderwiesen et al., 1978; Wilesmith, 1979) or large amounts of blood were transferred, as in premunization (Hugoson et al., 1968; Hugoson and Brattstrom, 1980; Marin et al., 1978; Stamatovic and Jonavic, 1968). Even though it has been demonstrated that 2500 washed lymphocytes, or 0.0005 ml of blood, are capable of infecting an animal (Van Der Maaten and Miller, 1978b), presence of serum neutralizing antibodies may reduce the infectivity

of an inoculum. In conclusion, data from this study did not suggest transmission of BLV infection at the time of vaccination for other infectious diseases. This may have resulted from an insufficient infective dose carried by a needle or from passive or nonspecific immunity protecting an animal from a potentially infective dose. Additional factors, such as the prevalence rate at the time of inoculation and age of donors, may further influence transmission.

Table IX-1. Summary of prevalence rates and frequency of detection of bovine leukemia virus (BLV) infection as related to three vaccination procedures.

Vaccination	Number vaccinated (1)	Mean age (days)	Number known to be infected with BLV at vaccination (2)	BLV prevalence rate (%) (2) ÷ (1)	Number detected with BLV	
					90 days prior to vaccination	90 days after vaccination
Leptospirosis	23	116	2	8.7	0	1
Brucellosis	186	131	14	7.5	8	3
IBR/BVD/PI$_3$*	61	326	8	13.1	1	8
Total	270		24	8.9	9	12

*Infectious bovine rhinotracheitis/bovine virus diarrhea/parainfluenza-3.

Table IX-2. Frequency of detection of bovine leukemia virus (BLV) infection before and after vaccination for other infectious diseases.

Period of observation	Number of animals	
	Detected with BLV infection	Remaining undetected for BLV infection
90 days after vaccination	12	250
90 days before vaccination	9	261

$$\text{Relative risk (RR)} = \frac{12(9+261)}{9(12+250)} = 1.38 \quad (p = 0.46)$$

Table IX-3. Frequency of detection of infection with bovine leukemia virus (BLV) in two independent groups of animals observed before and after vaccinations for other infectious diseases.

Observation period	Number of animals		
	Detected with BLV infection	Remaining undetected for BLV infection	Total
90 days before vaccination for viral diseases*	1	52	53+
90 days after vaccination for brucellosis	6	120	126

*Infectious bovine rhinotracheitis, bovine virus diarrhea, and parainfluenza-3.

+Fisher's exact $p = 0.33$.

CHAPTER X
SUMMARY

A prospective, longitudinal, observational design was followed in this epidemiologic study to examine for associations between bovine leukemia virus (BLV) infection and host and environmental factors. In this type of natural experiment, tests of hypotheses must be performed within the confines of such limitations as a dichotomous response variable, censoring, repeated measures, non-normality of data, and small sample sizes. Statistical methods were applied which specifically dealt with these analytic limitations. This methodology is applicable and appropriate for similar epidemiologic investigations of other infections and diseases.

Two epidemiologic tools were developed to improve estimates of time at infection based on serologic responses. The first was a prediction model which described the normal limits of decay of BLV-colostral antibodies. This was used to detect BLV infection in calves less than six months of age. Application of this technique in control programs for BLV infection or other infectious diseases could reduce transmission by early detection and subsequent removal of infected animals. The other tool developed was a seroconversion algorithm which allocated probabilities of

infection to segments of time preceding seroconversion. This allows examination of factors associated with infection rather than seroconversion.

Four age-related phases of infection with BLV were described in cattle from birth to 27 months of age. Age-specific rates of infection were approximated by quantitative and qualitative serologic response criteria.

The first phase of BLV infection was that acquired in utero. This was estimated to occur in 6.4% of calves born to BLV-infected cows. In utero infection was not associated with age or parity of the dam or with breed. Bull calves had a higher rate of in utero infection than heifer calves, although the difference was not significant statistically. In utero infection was independent of the stage of gestation in which the dam was infected. Calves infected in utero posed no major threat of infection to calves penned next to them in the calf barn.

The second phase of infection was observed from birth to six months of age. Infection detected in this period may have represented in utero infection in some calves. Transmission did occur during this period as demonstrated by a prevalence rate of 15% in six-month-old calves born to noninfected cows.

Sporadic infection characterized the third phase from 6 through 16 months of age. This phase was followed by a sharp increase in rates of infection from 16 to 27 months of age.

Survival analyses of age-specific rates of detection of BLV infection for 473 calves indicated that these rates were independent of dam age, dam parity, dam BLV-status, breed, or BLV-status of colostrum consumed. Interactions were suggested for age and dam status, age and colostrum status, and age and breed. Masking of infection by colostral antibodies may explain the first two interactions, and management differences the breed interaction.

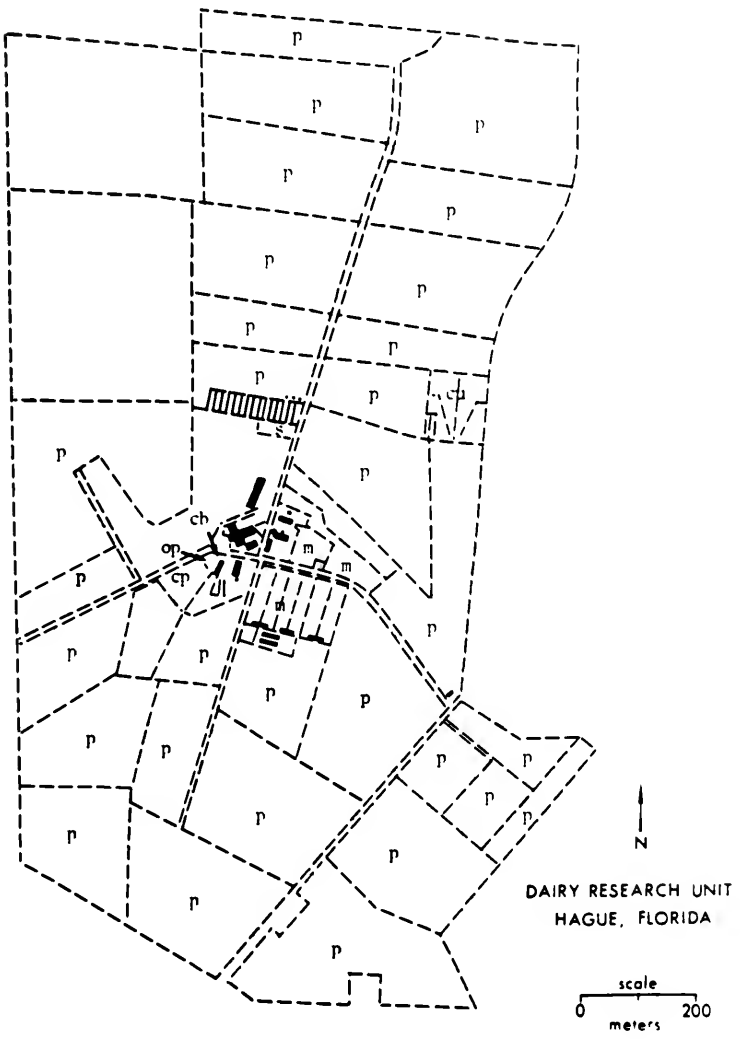
Management practices accounted for most of the variability in infection rates. Some types of locations occupied by the cattle were associated with lower risks of BLV infection. Individual outdoor calf pens and small calf pastures were associated with lower risks of infection relative to indoor calf pens and to the calf barn or paddocks, respectively. Very high risks of infection were associated with pasturing bred heifers with dry cows. Vaccination of calves and heifers for infectious diseases, however, did not appear to contribute to transmission of BLV infection.

Based on seasonal distributions of rates of BLV seroconversion, no evidence was found which supported arthropod vectors as a major mode of transmission in this population. However, a seasonal tendency was detected for infection in heifers over one year of age. This corresponded to movement of bred heifers into the dry herd in late winter and spring.

In conclusion, transmission of BLV infection in heifers prior to parturition could best be reduced by limiting exposure to older infected cattle. However, segregation of calves born to BLV-infected cows does not appear to be justified during the first two to three months of life. Use of the prediction model for colostral antibody decay can be used to identify young infected calves. Iatrogenic and vector-borne transmission did not appear to contribute to BLV infection in this cattle population.

Plat of the University of Florida Dairy Research Unit; m = small pastures used as maternity areas and as holding pens for mastitic and fresh cows; cb = calf barn with indoor pens; op = area of outdoor calf pens; cp = small calf pasture; cu = calf unit comprised of 5 small pastures; p = large pastures used by heifers, the dry herd, or lactating cows; s = small field containing sheep and goats.

APPENDIX A
PLAT OF THE UNIVERSITY OF FLORIDA DAIRY RESEARCH UNIT



APPENDIX B
AVERAGE MONTHLY HIGH AND LOW TEMPERATURES AND RAINFALL BETWEEN JULY 1, 1979, AND SEPTEMBER 30, 1981, FOR GAINESVILLE, FLORIDA.*

Month	Temperature (C)		Rainfall (cm)
	High	Low	
January	18.91	3.91	6.32
February	20.79	6.17	10.27
March	24.73	9.99	8.27
April	29.31	13.57	6.07
May	31.28	16.70	7.51
June	34.26	21.07	11.29
July	33.97	22.48	13.51
August	33.36	21.72	8.09
September	31.99	20.84	12.40
October	28.59	15.04	1.54
November	24.12	10.79	3.49
December	20.19	6.75	8.09

*Compiled from data received from the United States Department of Commerce, National Oceanographic and Atmospheric Administration, Asheville, NC.

APPENDIX C
MONTHLY FREQUENCIES OF CALVES BORN ALIVE BETWEEN JULY 1, 1979, AND JUNE 30, 1980, AT THE UNIVERSITY OF FLORIDA DAIRY RESEARCH UNIT.

Month of birth	Number	Percent
January	36	8
February	23	5
March	11	2
April	6	1
May	7	2
June	40	9
July	45	10
August	54	12
September	68	15
October	66	15
November	50	11
December	45	10
Total	451	100

APPENDIX D
LOCATION SITES AT THE DAIRY RESEARCH UNIT

Fig. D-1. Cow, fitted with udder bag, due to calve in a maternity pen.

Fig. D-2. Month-old calves in elevated pens in the calf barn.

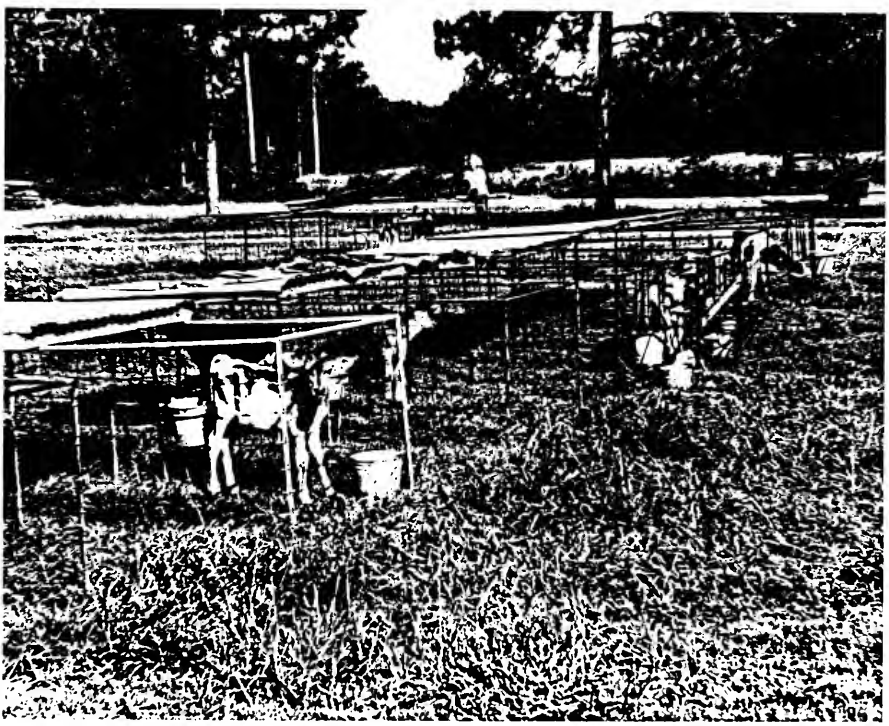


Fig. D-3. Month-old calves in individual outdoor calf pens.

Fig. D-4. Calves in paddocks adjacent to the calf barn.

Fig. D-5. Calves 3-4 months of age on small pastures (calf unit).

Fig. D-6. Heifers grazing on large pastures.

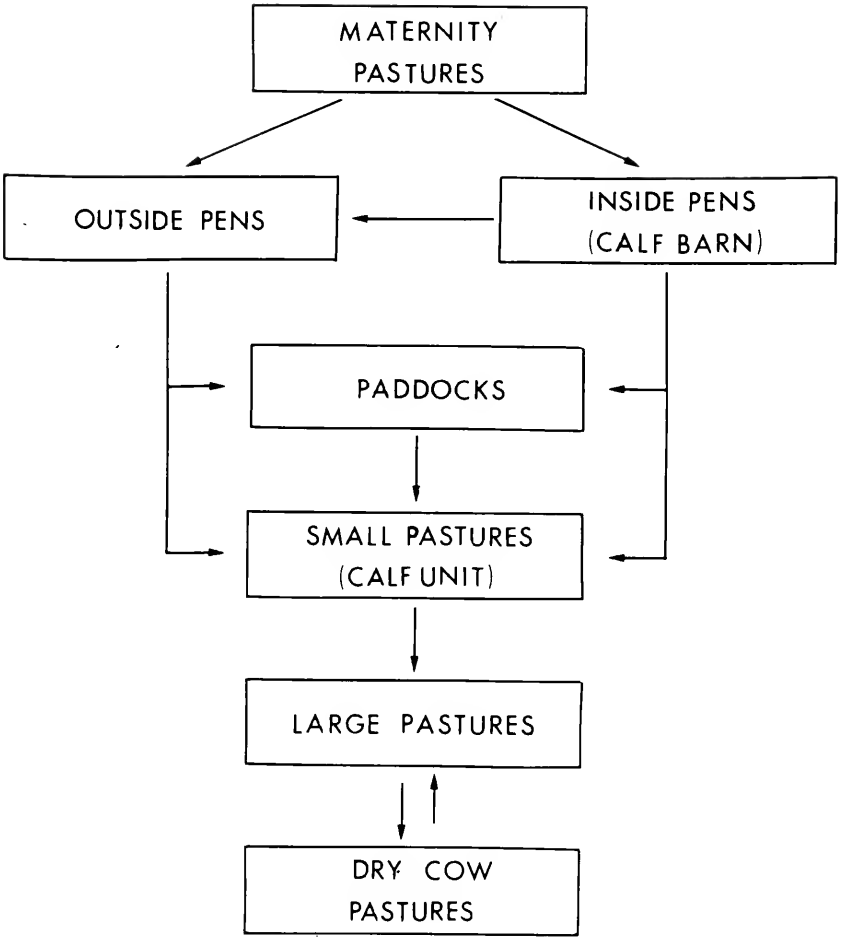
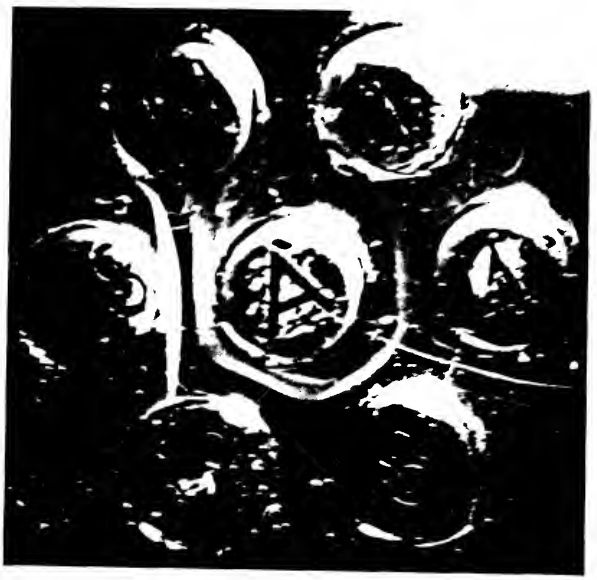
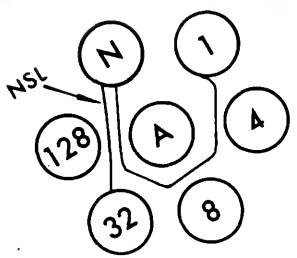


Fig. D-7. Flow chart of animal movements.

APPENDIX E
PRECIPITATION LINES OF AGAR-GEL IMMUNODIFFUSION





A = bovine leukemia virus glycoprotein-51 antigen; N = negative control serum; 1 = antibody titer of 1:1; 4 = antibody titer of 1:4; 8 = antibody titer of 1:8; 32 = antibody titer of 1:32; 128 = antibody titer of 1:128; NSL = nonspecific line.

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BIOGRAPHICAL SKETCH

Mark Cy Thurmond was born on February 19, 1947, in Seattle, Washington. He graduated from high school in Eureka, California, in 1965. From 1965 through 1972, he attended the University of California at Davis where he received a Bachelor of Veterinary Science degree in 1970 and a Doctor of Veterinary Medicine degree in 1972. During high school and college he was employed as a carpenter, mechanic, and hired ranch hand. He also raised registered Herefords and Shorthorn heifer replacements.

After receiving the DVM, he was a private practitioner on the northern California coast. In 1974 he returned to the University of California at Davis where he earned a master's degree in preventive veterinary medicine. His research examined immunological and meteorological factors involved in neonatal calf mortality.

From 1976 through 1977 he was a UN consultant to PAHO/WHO in Guyana, South America. Responsibilities as a consultant included curricula establishment, teaching, and administration in a regional Caribbean school to train animal and public health assistants. After returning to the U.S. in 1977, he resumed private practice in a large dairy area in California. In 1978 he married his wife,

Audrey, a botany student in Seattle. Between 1978 and 1979 he was a research veterinarian for the University of California. While there, he developed programs for computer surveillance of bovine mastitis, leptospirosis prophylaxis, and prevention of neonatal calf mortality. Since 1979, he has been engaged in graduate studies with emphasis on analytic epidemiology at the University of Florida.

He intends to continue teaching and research in applied field epidemiology of food animals.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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